Proceedings of the 42nd Annual Canadian Ecotoxicity Workshop: October 4-7, 2015, Saskatoon, Saskatchewan

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Comptes rendus du 42e atelier canadien annuel sur l'écotoxicité : du 4 au 7 octobre 2015, Saskatoon, Saskatchewan

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PROCEEDINGS OF THE 42ND ANNUAL CANADIAN ECOTOXICITY WORKSHOP: OCTOBER 4-7, 2015, SASKATOON, SASKATCHEWAN

Editors:

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Preface

For 41 years, the annual Aquatic Toxicity Workshop (ATW) has been held in various locations across Canada. In 2015, the ATW was rebranded as the annual Canadian Ecotoxicity Workshop (CEW) to reflect the broad scope of environmental interests held by workshop participants.

The 42nd annual CEW was held at the Radisson Hotel in Saskatoon, Saskatchewan from October 4 to 7, 2015. The workshop included an interactive panel session, 149 platform presentations and 44 poster presentations. Total attendance was 298.

This workshop was one of a continuing series of annual workshops in Canada on ecological toxicology, covering topics from basic aquatic toxicology to applications in environmental monitoring, setting of regulations and guidelines, and the development of sediment and water quality criteria. These workshops emphasize an informal exchange of ideas and knowledge on the topics among interested persons from industry, governments and universities. They provide an annual focus on the principles, current problems and approaches in ecotoxicology. These workshops are administered by a Board of Directors and organized by local organizing committees. The Proceedings are published with the support of Fisheries and Oceans Canada.

Préface

Pendant 41 années, l'Atelier annuel sur la toxicité aquatique (ATW) a eu lieu à divers endroits autour du Canada. En 2015, l'atelier a été rebaptisé l'Atelier canadien annuel sur l'écotoxicité (CEW) pour tenir compte de l'étendue des intérêts environnementaux généraux des participants à l'atelier.

Le 42ième Atelier canadien annuel sur l'écotoxicité a eu lieu à l'Hôtel Radisson à Saskatoon (Saskatchewan), du 4 au 7 octobre 2015. L'atelier a donné lieu à un panel interactif, 149 présentations orales et 44 présentations par affiche. Deux-cent quatre-vingt-dix-huit personnes ont assisté à l'atelier.

L'atelier a permis de poursuivre les discussions tenues annuellement au Canada sur l'écotoxicologie. Ces ateliers annuels organisés par un comité national constitué légalement réunissent des représentants des secteurs industriels, des administrations publiques et des universités que le domaine intéresse. Ces derniers y échangent des idées et des connaissances sur les notions fondamentales de la toxicologie aquatique, mais aussi sur son application pour la surveillance de l'environnement, l'élaboration de lignes directrices et de règlements, et la définition de critère pour les sédiments et pour la qualité de l'eau. Ils passent également en revue les principes de la spécialité, de même que les questions d'actualité et les méthodes adoptées dans le domaine. Les comptes rendus sont publiés avec le soutien de Pêches et Océans Canada.

Editors' comments

This volume contains papers, abstracts or extended abstracts of all presentations at the workshop. An author index is also included. The papers and abstracts were subject to limited review by the editors but were not subjected to full formal or external review. In most cases, the papers are published as presented and therefore are of various lengths and formats. Comments on any aspects of individual contributions should be directed to the authors. Any statements or views presented here are totally those of the speakers and are neither condoned nor rejected by the editors. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

The editors would like to thank Dr. Jill Watson for her assistance in preparing these proceedings.

Remarques des éditeurs

Ce compte rendu renferme le texte intégral ou le résumé de toutes les communications présentées aux ateliers. Un index des auteurs est aussi inclus. Les communications et les résumés ont été revus sommairement par les éditeurs, mais ils n'ont pas fait l'objet d'une revue exhaustive en bonne et due forme ou d'une revue indépendante. La longueur et la forme des communications varient parce que ces dernières sont pour la plupart publiées intégralement. On est prié de communiquer directement avec les auteurs pour faire des remarques sur les travaux. Toutes les déclarations et opinions paraissant dans le présent rapport sont celles des conférenciers; elles ne sont ni approuvées, ni rejetées par les éditeurs. La mention de marques de commerce ou de produits commercialisés ne constitue ni une approbation, ni une recommandation d'emploi.

Les rédacteurs voudraient remercier Dre. Jill Watson dans la préparation de ces comptes rendus.

42nd Canadian Ecotoxicity Workshop Organizing Committee / Comité organisateur du 42e atelier canadien sur l'écotoxicité

Workshop Co-Chairs / Co-présidents de l'atelier:

Karsten Liber	University of Saskatchewan, Toxicology Centre
David Janz	University of Saskatchewan, Toxicology Centre

Workshop Organizing Committee / Comité organisateur de l'atelier:

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Table of Contents / Table des Matières

PL – Platform presentation
PO – Poster presentation

Dr. Richard C. Playle Awards for Outstanding Theses in Ecotoxicology 1

Rainbow trout (*Oncorhynchus mykiss*) thrombocytes and B cells show similar expression of cytochrome, AhR, and P53 mRNA following *in vivo* exposure to benzo[a]pyrene (PL) 1
Laura J. Phalen, Bernd Köllner, Natacha S. Hogan and Michael van den Heuvel

Contaminants and Ecosystem Health 2

How much fish mortality are Environmental Effects Monitoring lethal fish population monitoring programs causing and is this a concern in small systems? (PL) 2
Cassandra Rees, Kelly Wells and Jennifer Doucette

Strengthening environmental toxicology capacity in developing countries: Ghana case example (PL)... 3
Nil Basu, Julius Fobil and Thomas Robins

Who and what reflects ecosystem health? A case study based on the oil sands (PL) 3
Judit Smits

Health status of fishes from the Athabasca and Slave River system, northern Canada (PL) 4
Paul Jones, Allison Hill, Brett Tendler, Ehimai Ohiozebau, Erin Kelly and John Giesy

Short-term recovery of benthic macro invertebrate assemblages from a Jet A-1 fuel spill in Lemon Creek, British Columbia (PL) 5
Cory Bettles, Cliff Robinson and Jason Baird

Effects of agricultural intensity and pesticide use on abundance of six aerial insectivorous bird species over 30 years in Canada's Prairie Pothole Region (PL)... 6
Nicole Michel, Robert Clark, Keith Hobson and Christy Morrissey

Spatio-temporal variation of metal concentrations in wetlands and amphibians from the oil sands region of northern Alberta, Canada (PL) 6
Fardausi Akhter, Danna Schock, Lukas Mundy, Catherine Soos and Bruce Pauli

Adaptive management in Canada's Northwest Territories: A case study of the application of prediction intervals to quantify natural variability (PL)... 7
Rainie Sharpe, Timothy Barrett, Hilary Machtans, Peter Chapman and Alexandra Hood

Contamination and multi-decadal decay of reservoir-liberated mercury in a downstream fishery: Effects of fish trophic level, size and age (PL) 8
Derek Green, Mark Duffy, David Janz, Kevin McCullum, Gary Carriere and Tim Jardine

Recovery of a mining-influenced stream ecosystem (PL) 8
Brian Fraser, Chris Mebane, and William Adams

Mercury biomagnification through lake food webs with different top predator fishes (PL).................... 9
Karen Kidd, Monica Finley, Meredith Clayden, Gretchen Lescord and Nelson O'Driscoll

Contaminants in the Arctic terrestrial ecosystem (PL).. 10
Mary Gamberg

Bioaccumulation of pharmaceuticals and personal care products in freshwater mussels *(Lasmigona costata)* in a river receiving wastewater effluent (PL) 10
Shane de Solla, Ève Gilroy, Joel Klinck, Laura King, Rodney McInnis, John Struger, Sean Backus and Patricia Gillis

Synthesizing spatiotemporal patterns and ecological drivers of neonicotinoid fate in Canadian Prairie Pothole wetlands (PL).. 11
Anson Main, Nicole Michel, John Headley, Kerry Peru, Allan Cessna and Christy Morrissey

Overview of DFO's National Contaminants Advisory Group: Assessing biological effects of contaminants on aquatic ecosystems (PO) 12
Bradley Park, Bronwyn Keatley, Bev Ross, Alain Dupuis, Cory Dubetz and Judith Leblanc

Analysis of hydrological parameter effects on organohalogenated micropollutant (polybrominated diphenyl ethers and polychlorinated biphenyls) behaviour: Case study of the Seine River in France (PO) 13
Khaoula Tlili, Pierre Labadie, Fabrice Alliot, Catherine Bourges, Annie Desportes and Marc Chevreuil

Factors affecting amphipod communities in estuaries of the Southern Gulf of St. Lawrence: Are contaminants affecting crustacean survival? (PO)... 13
Kyle Knysh, Michael Coffin, Simon Courtenay and Michael van den Heuvel

General Aquatic Toxicology.. 15

Can a bioaccumulation saturation model derived for the benthic invertebrate *Hyalella azteca* be a good predictor of uranium toxicity under natural conditions? (PL) 15
Lara Alves Beese and D. George Dixon

Chronic nickel and copper toxicity in the cryptic *Hyalella azteca* species complex (PL) 16
Patrick Pathammavong, Jonathan Witt, D. George Dixon and Warren Norwood

Effects of mercury on the health of wild fish populations in Kejimkujik National Park, Nova Scotia, Canada (PL).. 16
Stephanie Graves, Karen Kidd and Kelly Munkittrick

Developing the brook stickleback (*Culaea inconstans*) as a novel bioindicator for exposure to endocrine disrupting compounds using (anti-)androgen and estrogen responsive biomarkers (PL)..... 17
Breda Muldoon and Natacha Hogan

Acute cardiac effects of sublethal injections of benzo[a]pyrene and 5-azacytidine in juvenile rainbow trout (*Oncorrhyncus mykiss*) (PL) 18
Fred Leal

Sublethal effects of polymer-coated titanium dioxide nanoparticles and ultraviolet light co-exposure in developing zebrafish (*Danio rerio*) (PL) 19
Lindsey Felix and Greg Goss

Inferring long-term algal dynamics in prairie reservoirs (Saskatchewan, Canada) through environmental DNA in cores of sediments (PL) 19
Timothy Tse, Markus Hecker, Lorne Doig, John Giesy, Howard Wheater and Paul Jones

Is the development of the innate immune system in *Xenopus laevis* regulated by thyroid hormone? (PL) 20
Melanie Gallant and Natacha Hogan

Investigating the potential effects of pollutants on the gastrointestinal microbiome of rainbow trout (*Oncorhynchus mykiss*) (PO) 21
Ashley Moate, Tim Jardine, Steve Wiseman, Andrew Van Kessel and Markus Hecker

Determination of acute and sub-chronic toxicity of emerging contaminants to early life stages of rainbow trout (*Oncorhynchus mykiss*) (PO) 22
Dayna Schultz, Song Tang, Shawn Beitel, Bryan Sarauer, Sara Hanson, John Giesy, David Janz, Steve Wiseman, Paul Jones and Markus Hecker

Chemodynamic behaviour of thallium in the Slave River, Northwest Territories, Canada (PO) 23
Brett Tendler, Allison Hill, Ehimai Ohiozebau, John Giesy and Paul Jones

Direct and indirect toxicity of single-walled carbon nanotubes to freshwater invertebrates (PO) 24
Messika Revel, Michel Fournier and Pierre Yves Robidoux

Presence and toxicity of microplastics in the Canadian aquatic environment: A review (PO) 25
Julie Anderson, Bradley Park and Vince Palace

Predicting energy content and fish condition of juvenile rainbow trout (*Oncorhynchus mykiss*) using bioelectrical impedance analysis (PO) 25
Jason Durante, John Guchardi and Douglas Holdway

Predicting the bioaccumulation of organic sunscreen agents in rainbow trout (*Oncorhynchus mykiss*) using measured *in vitro* biotransformation rates (PO) 26
Leslie Saunders, Simon Fontanay and Frank Gobas

Pollution-induced effects in feral fish from the river Holtemme assessed by biomarker analysis (PO). 27
Nele Markert, Björn Deutschmann, Beat Thalmann, Shawn Beitel, Bryanna Eisner, Henner Hollert, Werner Brack and Markus Hecker

In vitro characterization of the toxicity of selected emerging contaminants to lake trout (*Salvelinus namaycush*) (PO) 28
Norina Pagano, Shawn Beitel, Bryanna Eisner, Burkhard Schmidt, Henner Hollert and Markus Hecker

Evaluation of genotoxic substances in zebrafish embryos using the fish embryo toxicity and micronucleus assays (PO) 28
Lilli Senn, Carolina Di Paolo, Thomas-Benjamin Seiler, Henner Hollert, Shawn Beitel, Bryanna Eisner and Markus Hecker

Mechanisms affecting arsenic flux rates from prairie reservoir sediments, Buffalo Pound Lake, Saskatchewan, Canada (PO) 29
Lawrence D'Silva, Karsten Liber, Helen Baulch and Lorne Doig

Mitigating chronic arsenic poisoning in a Bangladeshi community using high-selenium lentils: Planning and design of a clinical trial (PO) 30
Regina Krohn, Evana Akhter, Albert Vandenberg, Rubhana Raqib and Judit Smits

Development of a novel biological marker of endocrine disruption in rainbow trout (PO) 31
Yuxiu Chen, M. Soron, B. Hersikorn, A. Matthews and Jason Raine[1]

In vivo effects of intravenously injected polymer-coated TiO_2 nanoparticles on goldfish (*Carassius auratus*) immunity (PO) 32
Van Ortega, David Boyle, James Stafford and Greg Goss

Selenium Ecotoxicology 34

Evaluation of selenium sensitivity of spotted sandpipers breeding in the Elk River watershed of southeastern British Columbia (PL) 34
Shari Weech, Patti Orr, Carla Fraser and Kirsten Gillespie

Concentration dependence and interspecific differences in selenium partitioning between fish tissues (PL) 35
Adrian deBruyn and Emily-Jane Costa

What do we know about the effects of dietary selenomethionine on white sturgeon (*Acipenser transmontanus*)? (PL) 35
Jenna Zee, Sarah Patterson, Danielle Gagnon, Markus Hecker

Assessing the potential effects of chronically elevated dietary selenium exposure on the fitness of the model amphibian *Xenopus laevis* (PL) 36
Anita Massé, David Janz and Jorgelina Muscatello

Community composition matters: Selenium accumulation in periphyton and trophic transfer to the freshwater snail, *Stagnicola elodes* (PL) 37
Lorne Doig, Vanessa Pittet, Blue Markwart, Monique Haakensen and Karsten Liber

Legacy effects of coal combustion residuals on freshwater ecosystems in North Carolina (PL) 38
Jessica Brandt, Richard Di Giulio, Emily Bernhardt and Avner Vengosh

Effects of chronic dietary exposure to selenomethionine on the transcriptome of juvenile white sturgeon (PO) 39
Sarah Patterson, Jenna Zee, Song Tang, Steve Wiseman and Markus Hecker

Potential metabolic and cardiovascular effects of dietary selenomethionine exposure in adult zebrafish (*Danio rerio*) (PO) 39
Connor Pettem, Lynn Weber and David Janz

Assessing the fate and effect of dietary-Se on *Daphnia magna* using a modified two-generation life-cycle test (PO) 40
Carrie Rickwood, Jesse Rentz, Craig Johnson and Morgan King

Alternative Non-vertebrate Test Methods.. 41

An examination of selenium-cadmium interactions using primary cultures of rainbow trout (*Oncorhynchus mykiss*) hepatocytes (PL)..41
Ankur Jamwal and Som Niyogi

Development of sentinel species of benthic invertebrates for use in estuaries (PL) 41
Bryce Pippy, Karen Kidd and Kelly Munkittrick

Characterization of metallothionein in two ancient fishes exposed to metals: White sturgeon and lake sturgeon (PL) 42
Shawn Beitel, Jon Doering, Bryanna Eisner, Timon Heide, Henner Hollert, John Giesy, Markus Hecker and Steve Wiseman

Cross-species comparison of relative potencies and relative sensitivities of fishes to dibenzo-p-dioxins, dibenzofurans, and polychlorinated biphenyls *in vitro* (PL)..43
Bryanna Eisner, Jon Doering, Shawn Beitel, Steve Wiseman, Jason Raine and Markus Hecker

An animal testing strategy for industry: A Canadian perspective (PL)...43
Jonathan Naile, Sarah Hughes, Graham Whale, Charles Eadsforth and Chantal Smulders

Is something fishy with Environment Canada's adoption of animal alternatives? (PL)...........................44
Lisa Taylor and Rick Scroggins

Exploring right hand coiled coil (RHCC) proteins as a matrix for a new aquatic passive sampling device for PAHs (PO)..45
Vince Palace, Jorg Stetefeld, Matthew McDougall, Mark Hanson and Jennifer Leslie

Risk Assessment and Regulatory Toxicology.. 46

The case for deriving site-specific water quality objectives driven by science (PL)...............................46
Albert Shpyth, Don Hart and Ron Nicholson

Applicability of risk assessment and toxicology in regulating Alberta's Energy Resources (PL).............46
Mandy Dumanski

Developing nitrogen species limits for a mine effluent discharged to a non-fish-bearing stream (PL)..47
Meghan Goertzen and Robert Goldblatt

Assessing our standards: The effect of temperature on toxicity of metal salts to six Canadian cold-water fish species (PL)..48
Dana Moore, David Poirier, Paul Sibley and Keith Solomon

Ecological risk assessment at the former Cutler Acid Plant, Ontario: Approaches for evaluating risks to aquatic receptors in lake, stream, ditches and marshy areas (PL)..49
Heather Fenton, Nicole MacDonald, Kerrie Serben, Michael Z'Graggen, Roy Angelow, Sue-Jin An and Barbara Slezak

Ecological risk assessment at the former Cutler Acid Plant, Ontario: Assessing risks to ground-dwelling invertebrates in low-pH, metal-contaminated soil using a weight-of-evidence approach (PL) 50
Heather Fenton, Roy Angelow, Tammie Morgan-Gray, Nicole MacDonald, Michael Z'Graggen, Sue-Jin An and Barbara Slezak

Using saturation kinetics-based models to predict *Hyalella azteca* mortality in chronic zinc exposures (PL) 50
Lesley Milne Wilkinson, Warren Norwood and D. George Dixon

Large-scale risk assessment of organic chemicals in river ecosystems: A European perspective (PL) ... 51
Egina Malaj, Peter von der Ohe, Matthias Grote, Werner Brack and Ralf Schäfer

The EU project SOLUTIONS: Challenges and responses for present and future emerging pollutants in land and water resources management (PL) 52
Henner Hollert and Werner Brack

Is received dose from ingested soil independent of soil PAH concentrations: Animal model results (PO) 53
Rachel Peters, Mark Wickstrom and Steven Siciliano

Herbicides in Manitoba: Estimating environmental exposure and improving aquatic monitoring in the province (PO) 53
Dana Moore, Charles Wong and Mark Hanson

Sulfonamide antibiotic transformation products in wastewater effluent dominated streams pose little risk of toxicity to aquatic organisms (PO) 54
Chelsea Lobson and Mark Hanson

New Substances Program tools used to assess the quality of test data during ecological risk assessment (PO) 55
Cristina Inglis

Ecological screening assessment of substances in the "Certain Organic Flame Retardants" grouping under the Government of Canada's Chemical Management Plan (PO) 55
Jenny Ferone, John Pasternak, Michael Beking, Vincent Dorais, Barbara Elliott, Magdalena Jagla, Jen-ni Kuo and Kaiqi Yao

Behavioural Ecotoxicology 57

Use of behavioural endpoints in developing U.S. Environmental Protection Agency Water Quality Criteria: Yes, no, or maybe so? (PL) 57
Burt Shephard

The role of water hardness in physiology and behaviour of freshwater organisms (PL) 57
Aditya Manek, Dylan Steinkey, Brett Dillman, Cindy Meays, Lynn Weber and Gregory Pyle

Assessing "sublethal" effects of acidification in freshwater habitats: Lessons from Atlantic salmon in the Little Southwest Miramichi River (PL) 58
Grant Brown and Chris Elvidge

The antidepressant venlafaxine is a neuroendocrine disruptor and affects stress response and behaviour in fish (PL) 59
Matt Vijayan, Nataliya Melnyk-Lamont, Carol Best and Andrew Thompson

Local adaptation of finescale dace (*Phoxinus neogaeus*) olfaction, behaviour, and swim physiology post-exposure to natural and anthropogenic sources of bituminous toxicants (PL) 60
Sylvia Chow and Greg Pyle

The impact of dilbit and conventional crude oil exposure on basal activity and visual learning behaviour in zebrafish (*Danio rerio*) (PL)....................61
Danielle Philibert, Clara Philibert, Carlie Lewis and Keith Tierney

The role of suspended particulate matter on the toxicity of oil sands process-affected water on feeding behaviour of *Daphnia magna* (PL)....................61
Ebrahim Lari, Effat Mohaddes and Greg Pyle

Behavioural methods in aquatic toxicology (PL)....................62
Robin Calfee, Edward Little and Holly Puglis

Acute and chronic exposure to treated wastewater effluent affects movement and aggression in round goby (PL)....................63
Erin McCallum

Multi-Generational Toxicology and Epigenetics....................64

Larval exposure to fluoxetine suppresses the stress response in adult zebrafish across one generation (PL)....................64
Marilyn Vera Chang, Thomas Moon and Vance Trudeau

Effects of the novel brominated flame retardant 1,2,5,6-tetrabromocyclooctane (TBCO) on reproductive capacity of 2 generations of Japanese medaka (PL)....................65
Steve Wiseman, Jianxian Sun, David Saunders, Jeremy Kuzma and John Giesy

Maternal transfer of bisphenol A disrupts the liver proteome of rainbow trout in multiple generations (PL)....................65
Matt Vijayan, Laura Dindia, Oana Birceanu and Brendan McConkey

Airborne exposure of polycyclic aromatic hydrocarbons (PAHs) in Hamilton Harbour, Ontario, affects global DNA methylation in wild double-crested cormorants (*Phalacrocorax auritus*) (PL)....................66
Sarah Wallace, Shane de Solla and Valerie Langlois

Genetic polymorphism and epigenetic evidence from animals and humans to advance the risk assessment of mercury (PL)....................67
Nil Basu

Role of DNA methylation in the regulation of CYP1A induction by polycyclic aromatic hydrocarbons (PO)....................67
Jonas Brandenburg and Jessica Head

Municipal Wastewater....................69

Characterization of the endocrine potencies of municipal effluents across Canada using *in vitro* bioassays (PL)....................69
Tabata Bagatim, Sara Hanson, Hongda Yuan, Kean Steeves, Steve Wiseman, Natacha Hogan, Alice Hontela, Paul Jones, John Giesy, Leslie Bragg, Hadi Dhiyebi, Mark Servos, Charles Gauthier, François Gagné and Markus Hecker

Thyroid hormones and thyroid hormone disruptors and their effects on chemosensation in North American bullfrog (*Lithobates catesbeianus*) tadpoles (PL) 70
Jody Heerema, Caren Helbing, Aditya Manek and Gregory Pyle

Reproductive and histopathological effects of municipal wastewater effluent exposure in male and female fathead minnows (PL) 71
Kean Steeves, Sara Hanson, Tabata Bagatim, Steve Wiseman, Paul Jones, John Giesy, Markus Hecker, Alice Hontela and Natacha Hogan

Assessing potential endocrine disrupting effects of municipal effluents on fathead minnow (*Pimephales promelas*) populations in Southern Saskatchewan (PL) 71
Sara Hanson, Tabata Bagatim, Kean Steeves, Steve Wiseman, Natacha Hogan, Alice Hontela, Paul Jones, John Giesy and Markus Hecker

Annual variability in biological measures in response to chemicals of emerging concern (PL) 72
Meghan Fuzzen, Gérald Tétreault, Leslie Bragg, Paulina Bahamonde, Rajiv Tanna, Mark McMaster and Mark Servos

Reduction in intersex incidence and severity in a wild fish population after infrastructure upgrades at a municipal wastewater treatment plant (PL) 73
Keegan Hicks, Meghan Fuzzen, Patricija Marjan, Leslie Bragg, Emily K. McCann, Gérald Tétreault, Mark McMaster and Mark Servos

Assessing the impact of wastewater effluents in the Grand River, Ontario (PL) 74
Mark Servos, Leslie Bragg, Hadi Dhiyebi, Meghan Fuzzen, Keegan Hicks, Patricija Marjan, Emily K. McCann, Maricor Arlos, Chris Martyniuk, Glen Van Der Kraak, Deborah MacLatchy, Gérald Tétreault and Mark McMaster

Endocrine disruption of emerging compounds in municipal wastewaters: Impacts in the St-Lawrence River (PL) 74
Sébastien Sauvé, Michel Fournier, François Gagné, Mélanie Desrosiers, André Lajeunesse, Gilbert Cabana, Patrice Couture, Émilie Lacaze, Charles Gauthier and Pauline Brousseau

Assessment of environmental risks related to contaminants in municipal wastewaters: A toolbox approach (PL) 75
Karen Kidd, Mark Servos, Markus Hecker and Francois Gagné

Using weight of evidence to evaluate potential effects of trace organics on aquatic life (PL) 76
John Wolfe, Carrie Turner, Doug Bradley, Allen Burton, Drew McAvoy and Jennifer Daley

Occurrence of wastewater contaminants at Norway House Cree Nation, Manitoba, Canada (PO) 77
Jonathan Challis, Kim Luong, Chelsea Lobson, Charles Knapp, Charles Wong and Mark Hanson

Transcriptome profiling in the liver of wild rainbow darter (*Etheostoma caeruleum*) in the central Grand River, Ontario, to assess impact and recovery of resident populations (PO) 77
Patricija Marjan, Deborah MacLatchy, Christopher Martyniuk and Mark Servos

Non-conventional Oil and Gas 79

In vitro and *in vivo* oxidative stress responses of oil sands process-affected water (PL) 79
Jianxian Sun, Hattan Harbi, Hui Peng, John Giesy and Steve Wiseman

Long-term effects in fathead minnow exposed to oil sands sediments early in life (PL) 80
Joanne Parrott, Caroline Vignet, Zhendi Wang, Chun Yang, Richard Frank, Mark Hewitt and Mark McMaster

Assessing the impact of source and age of oil sands process-affected water on the toxicity of extracted naphthenic acids (PL) 80
Adrienne Bartlett, Richard Frank, Patricia Gillis, Lisa Brown, Mark Hewitt, Pamela Brunswick, Dayue Shang and Joanne Parrott

Toxicity of aqueous vanadium to aquatic organisms relevant to the Athabasca Oil Sands region for use in development of water quality guidelines (PL) 81
Stephanie Schiffer, Lorne Doig and Karsten Liber

The influence of water chemistry on the acute aqueous toxicity and speciation of vanadium (PL)...... 82
Esteban Gillio Meina and Karsten Liber

Effects of diluted bitumen exposure on the cardiovascular system of juvenile sockeye salmon: From cells to performance (PL) 82
Sarah Alderman, Laura Dindia, Feng Lin, Anthony Farrell, Chris Kennedy and Todd Gillis

Response of native amphibians to reclaimed wetlands in the Athabasca Oil Sands (PL) 83
Jaimie Klemish, Sarah Bogart, Greg Pyle and Michael Lannoo

Exposure of fish in the Athabasca and Slave Rivers to PAHs potentially derived from petrogenic activities (PL) 84
Ehimai Ohiozebau, Brett Tendler, Gary Codling, Erin Kelly, John Giesy and Paul Jones

Investigations of oil sands influences on the Athabasca River Delta: PAHs, metals, and benthos (PL).. 85
Marlene Evans, Dirk de Boer, Martin Davies and Bruce Kilgour

Investigating the impacts of atmospheric emissions from Athabasca Oil Sands on adjacent boreal lakes through beta diversity patterns of zooplankton communities (PL) 86
M. U. Mohamed Anas, Buddhine Meegahage, Marlene Evans, Jonathan Keating, Dean Jeffries and Bjoern Wissel

Constraints on biodiversity and ecosystem functioning in the Athabasca oil sands: Using gamma irradiation to detoxify fine fluid tailings waste (PL) 87
Chantal Dings-Avery, Jan Ciborowski and Christopher Weisener

The complexity and challenges in assessment of toxicity of hydraulic fracturing flowback fluids (PL).. 88
Yuhe He, Erik Folkert, Shannon Flynn, Daniel Alessi, Jonathan Martin and Greg Goss

Effects of water accommodated fractions of a hydraulic lubricating oil on larval amphibians (PL)....... 89
D. Landon McPhee, Laura Halyk, Karl Bresee, Connie Semper, Steven Siciliano and Natacha S. Hogan

Short-term bioaccumulation of hydrocarbons by the blue mussel (*Mytilus edulis*) exposed to conventional and non-conventional crude oils spilled under ice-covered seawater (PL) 89
Richard Saint-Louis, Emilien Pelletier, Jean-Pierre Gagné, Céline Audet and Réjean Tremblay

Induction of EROD activity and oxidative stress in gill of rainbow trout (*Oncorhynchus mykiss*) exposed to hydraulic fracturing flowback fluid (PO) 90
Yuhe He, Erik Folkert, Shannon Flynn, Daniel Alessi, Jonathan Martin and Greg Goss

Assessing the mechanistic toxicity of Oil Sands process affected waters (OSPW) (PO) 91
Garrett Morandi, Steve Wiseman, John Giesy, Jon Martin, Alberto Pereira, Rishikesh Mankidy, Miao Guan and Xiaowei Zhang

Identification and evaluation of chemicals of potential concern to aquatic life in oil sands process-affected water from Base Mine Lake, Alberta (PO) 92
Kevin White and Karsten Liber

Effect of dissolved organic compounds from oil sands process-affected water (OSPW) on toxicity of 7-isopropyl-1-methylphenanthrene (retene) to early life stages of Japanese medaka (*Oryzias latipes*) (PO) 92
Hattan Alharbi

Acute effects of exposure to oil sands-impacted sediments on the early life stages of *Xenopus laevis* (PO) 93
Melanie Gallant, Nicole Baldwin, Julie Bilodeau, Jules Blais, Bruce Pauli and Natacha Hogan

Sediment and Soil Ecotoxicity 95

Broadening horizons: Investigating metal fate and effects in sediments (PL) 95
Carrie Rickwood and Philippa Huntsman-Mapila

Validation of a new standardized test method for the freshwater amphipod, *Hyalella azteca* (PL)...... 95
Lisa Taylor, Lesley Novak, Martina Rendas, Paula Antunes and Rick Scroggins

Mechanisms affecting arsenic flux rates from prairie reservoir sediments, Buffalo Pound Lake, Saskatchewan, Canada (PL) 96
Lawrence D'Silva, Karsten Liber, Helen Baulch and Lorne Doig

Tesseracts and toxicity: Metal mixture testing in soil (PL) 97
Steven Siciliano, Beverley Hale, Mark Cousins, Kayode Jegede, Kobby Awuah and Amanda Laird

Managing contaminant risks to soil-associated flora and fauna in Canada: Evolution of standardized toxicity test methods in contrast with the retrogression of contaminated sites practitioners? (PL) 98
Doug Bright

Ecological description and classification of contaminated and reference soil samples sites (PL) 98
Jason Nelson, Rick Scroggins and Mary Moody

A method for assessing contamination in boreal and northern wetland habitats using native plant species (PL) 99
Mary Moody and Rick Scroggins

Development of a new Environment Canada test method for measuring survival and reproduction effects in soil using the oribatid mite, *Oppia nitens* (PL) 100
Rick Scroggins, Ryan Hennessy, Juliska Princz, Muriel Jatar, Heather Lemieux, Leana Van der Vliet and Lisa Taylor

Can we predict polycyclic aromatic hydrocarbons bioavailability to mammals from incidentally ingested soils? (PL) 100
Steven Siciliano, Kyle James, Rachel Peters, Mark Cave, Mark Wickstrom and Eric Lamb

New Tier 1 Boron Guideline for Alberta: Boron soil ecotoxicity and analytical methods development research (PL) 101
Darlene Lintott and Gregory Huber

Sensitivity of freshwater invertebrates to sediment-derived substituted phenylamine antioxidants (PO) 102
Ryan Prosser, Danielle Milani, Adrienne Bartlett, Patty Gillis and Vimal Balakrishnan

Water Quality Benchmarks 103

Water Quality Guidelines in Canada: The meandering flow of the river! (PL) 103
Uwe Schneider

Using species sensitivity distributions to derive an arsenic water quality effects benchmark for aquatic ecological risk assessment (PL) 103
Kerrie Serben, James Dwyer and Adrian deBruyn

Field- and lab-based metal bioavailability and aquatic impact assessment at a closed gold mine (PL) 104
Helga Sonnenberg and Karen Munro

An evaluation of the effect of water hardness on chronic toxicity of cadmium in support of development of a Site-Specific Water Quality Objective (PL) 105
Brett Lucas, Ryan Hill, Emma Marcus, Karen Lee, Josh Baker, Yvonne Lam, Jesalin Wijaya and James Elphick

Understanding the effects of sulfate and hardness in mine seepage on an aquatic receiving environment (PL) 105
Martin Davies, James Elphick, John Wilcockson and Michelle DesJardins

Environmental Assessment and Cumulative Effects 107

Using Landsat imagery to backcast fire and post-fire residuals in the Boreal Shield of Saskatchewan: Implications for woodland caribou management (PL) 107
John Kansas, Javier Vargas, Hans Skatter, Brady Balicki and Kevin McCullum

Refining environmental impact assessments: Pathway analysis (PL) 107
Jennifer Fernet

Analysis of cumulative effects and determination of significance (PL) 108
John Virgl

The effect of historical discharge from the abandoned Giant Mine on slimy sculpin (PL) 109
Dave Huebert and Carey Sibbald

Investigation of Cause: Tools for assessing habitat quality *vs.* metal exposure using a weight of evidence approach (PL) 109
Lisa Rozon-Ramilo, Helga Sonnenberg, Joel Nilsen and Joe Keene

Peel River watershed: Temporal trends in hydrology and water chemistry (PL)................................ 110
Dave Huebert, Carey Sibbald and Mike McKernan

Future directions for using natural variability to develop triggers for designing and adapting environmental monitoring programs for cumulative effects assessment (PL).................................... 111
Kelly Munkittrick, Tim Arciszewski, Bruce Kilgour, Keith Somers and Tim Barrett

Incorporating the duty to consult and traditional knowledge into environmental assessments (PO). 112
Katie Zdunich, Brent Murphy and Phil Anaquod

Environmental Assessment, Protection and Management for Uranium Mining and Milling. 113

Environmental performance of a theoretical modern uranium mine/mill: Based on a 13-year monitoring dataset (PL).. 113
Malcolm McKee, Elias Dagher, Nana-Owusua Kwamena and Barbara Dowsley

The evolution and contribution of environmental performance reporting for uranium mines and mills in Saskatchewan (PL).. 114
Janeen Tang, Lynnae Dudley and Al Shpyth

Potential impacts following an accidental release during transportation (PL)................................... 114
Stacey Fernandes, Mehran Monabbati, Dale Huffman and Sarah Benson

Effects of uranium mining on benthic invertebrate communities in the Athabasca Basin of Northern Saskatchewan (PL).. 115
Bruce Kilgour, Barbara Dowsley, Malcolm McKee and Steve Mihok

Investigation of cause for a Northern Saskatchewan mine (PL).. 115
James Elphick, Josh Baker, Charlene Burnett-Seidel and Howard Bailey

Factors influencing polonium-210 bioaccumulation in resident biota and caged oligochaetes in receiving environments downstream of closed uranium mines (PL)... 116
Cheryl Wiramanaden, Patti Orr, Kim Connors and Cynthia Russel

Effects of chronic exposure to radium-226 on health and reproductive fitness of mice (PL).............. 116
Marilyne Stuart, Stephanie Walsh, Meloja Satkunam, Ben Su, Amy Festarini, Michelle Bugden, Harry Peery and Carmel Mothersill

Overview of a country foods study conducted near the decommissioned Beaverlodge uranium mine and mill site in northern Saskatchewan (PL)... 117
Samantha Pham and Ryan Froess

Risk evaluation of the country foods study conducted near the decommissioned Beaverlodge uranium mine and mill site in northern Saskatchewan (PL)... 118
Harriet Phillips, Leah Leon, Stacey Fernandes and Mike Webster

Bioavailability of sediment-associated uranium to the freshwater midge, *Chironomus dilutus*: Lessons learned from sediment physicochemical properties (PO).. 118
Sarah Crawford and Karsten Liber

Wildlife Ecotoxicology and Disease **120**

A spatial and epidemiological analysis of deformed amphibians in southern Québec, Canada: What do deformed frogs tell us about the state of the environment? (PL) 120
Bruce Pauli, Jean Rodrigue, Isabelle Gauthier and Martin Ouellet

Sublethal effects of neonicotinoid pesticides on wood frogs: Mesocosm exposures (PL) 121
Stacey Robinson, Stacey Lee-Jenkins, Sarah Richardson, Vance Trudeau and Bruce Pauli

Forty-year decline of organic contaminants in eggs of herring gulls (*Larus argentatus*) from the Great Lakes, 1974 to 2013 (PL) 122
Shane de Solla, Chip Weseloh, Kimberley Hughes and Dave Moore

Identifying effects of wintering area on contaminants in White-winged Scoters: An isotopic approach (PL) 122
Kirsty Gurney, Cindy Wood, Ray Alisauskas and Stuart Slattery

Methylmercury metabolism across six Arctic marine mammals: Interspecies differences in exposure and neurotoxic risk (PL) 123
Nil Basu, Christian Sonne and Rune Dietz

S.W.O.T. analysis of the search for sentinels of exposure to air pollution (PL) 124
Michelle North, David Kinniburgh and Judit Smits

Investigating whether industrial pollutant exposure affects the pre-migratory fuelling ability and the migration performance of two shorebird species (PL) 125
Kristin Bianchini, David Newstead and Christy Morrissey

Selenium toxicity in wildlife: Looking beyond fish (PL) 125
Mariah Arnold and Jorgelina Muscatello

Ecology and opportunities to study ecotoxicology of woodland caribou, wolves, and black bears in the Saskatchewan Boreal Shield (PO) 126
Philip McLoughlin

Relationship between body condition and contaminants in overwintering canvasbacks (*Aythya valisineria*) in the Lake St. Clair/St. Clair River Area (PO) 127
Shane de Solla, Kimberley Hughes, Pamela Martin, Michael Schummer and Scott Petrie

Bioremediation and Reclamation **128**

Biogeochemical instability of oil sands composite tailings undergoing reclamation (PL) 128
Lesley Warren

Tracking microbial biomarkers in contaminated environments and bioremediation (PL) 129
Christopher Weisener

In situ bioremediation of saturated mine materials (PL) 129
James Harrington

What is the fate of a lubricating oil in Canadian soils? (PL) 130
Amy Gainer and Steven Siciliano

Treatment of contaminated waters using a phased process-driven design of Constructed Wetland Treatment Systems (PL) 131
Monique Haakensen and Vanessa Pittet

Variation in invertebrate community composition in a constructed wetland along environmental gradients (PO) 131
Kellie Menard, Mallory Hazell, A. Lee Foote and Jan Ciborowski

Applications of microbial profiling for optimized bioremediation strategies (PO) 132
Vanessa Pittet and Monique Haakensen

Predictive Ecotoxicology / Adverse Outcome Pathways 133

Derivation and evaluation of adverse outcome pathways for the effects of cyclooxygenase inhibitors on reproductive processes in fish (PL) 133
Dalma Martinovic-Weigelt, Alvine Mehinto, Gerald Ankley, Jason Berninger, Timothy Colette, John Davis, Nancy Denslow, Elizabeth Durhan, Evan Eid, Drew Ekman, Kathleen Jensen, Michael Kahl and Daniel Villeneuve

The stoichiometric pathway analysis of steroidogenesis in fathead minnows (*Pimephales promelas*) (PL) 134
David Hala

Understanding the effects and mode of action of emerging contaminants (benzotriazoles) using RNA-sequencing in *Daphnia magna* (PL) 134
Maeva Giraudo, Mélanie Douville, Guillaume Cottin and Magali Houde

Characterization of toxicity pathways of 2,3,7,8-TCDD, PCB 77, and benzo[a]pyrene in white sturgeon using whole transcriptome and proteome analyses (PL) 135
Jon Doering, Song Tang, Steve Wiseman, Hui Peng, Bryanna Eisner, Jianxian Sun, John Giesy and Markus Hecker

Utilizing high throughput bioassays to characterize the bioactivity of complex environmental samples (PL) 136
Brett Blackwell, Anthony Schroeder, Keith Houck, Gerald Ankley and Daniel Villeneuve

The brominated flame retardant TBCO affects fecundity and transcript profiles of the HPGL-axis in Japanese medaka (PO) 137
David Saunders, Steve Wiseman and John Giesy

Pesticides 138

Green eggs and atrazine (PL) 138
Mark Hanson, Leilan Baxter, Richard Brain, Alan Hosmer and Keith Solomon

Acute effects of imidacloprid and monocrotophos on migratory behaviour in white-throated (*Zonotrichia albicollis*) and white-crowned sparrows (*Zonotrichia leucophrys*) (PL) 138
Leanne Flahr, Bridget Stutchbury and Christy Morrissey

Teratogenic and behavioural effects of imidacloprid on honey bees (*Apis mellifera*) (PL) 139
Sarah Wood, Madeleine Friesen, Gary Wobeser and Elemir Simko

Assessing the acute and chronic toxicity of neonicotinoid insecticides to non-target aquatic species (PL) 140
Adrienne Bartlett, Amanda Hedges, Patricia Gillis, Lisa Brown, Rodney McInnis, John Struger and Shane de Solla

Impacts of neonicotinoid insecticides on aquatic insect emergence in prairie wetlands using *in situ* limnocorrrals (PL) 141
Michael Cavallaro, Christy Morrissey, John Headley, Kerry Peru and Karsten Liber

Neonicotinoid insecticide mixtures: Characterizing cumulative toxicity under acute exposure scenarios (PL) 141
Erin Maloney, Christy Morrissey, John Headley, Kerry Peru and Karsten Liber

Sublethal effects of the neonicotinoid imidacloprid on developing sockeye salmon (*Oncorhynchus nerka*) (PO) 142
Vicki Marlatt, Genvieve St-Martin, Sean Engelking, Sara Modares and Chris Kennedy

Persistence and toxicity of Garlon XRT® and Arsenal Powerline® in soils along transmission right-of-ways in the Yukon Territory (PO) 143
Amy Jimmo, Katherine Stewart and Steven Siciliano

Metal Mixture Toxicity 144

Characterizing multiple metals (Ag, Cd, Cu, Ni, Pb and Zn) interactions on metal uptake in the freshwater snail, *Lymnaea stagnalis* (PL) 144
Anne Crémazy, Kevin Brix and Chris Wood

Effects of waterborne metals in binary mixtures on short-term gill metal-binding and ion uptake, and toxicity in rainbow trout (*Oncorhynchus mykiss*) (PL) 145
Som Niyogi, Sunita Nadella and Chris Wood

Considerations for assessing multi-metal toxicity in fish: Effects of lead and cadmium mixtures on gill function (PL) 145
Michael Wilkie and Yvonne Clemow

Toxic outcome drastically altered after exposure to a four-way mixture of Cd, Se, SO_4, and NO_3 in species-specific water (PL) 146
Sarah Bogart, Cindy Meays and Greg Pyle

Nickel and zinc protect against cadmium-induced olfactory toxicity in rainbow trout (PL) 147
Greg Pyle and Bill Dew

Evaluating the interactive effects of waterborne metals (Cd, Cu, Ni and Zn), singly and in binary combination, on fathead minnow (*Pimephales promelas*) reproductive performance (PL) 148
Melissa Driessnack, Ankur Jamwal and Som Niyogi

Industrial Development: Implications for Aboriginal Communities 149

The importance of community-based environmental monitoring programs in regions containing industrial developments (PL) 149
Ryan Froess, Cassandra Rees, Kelly Wells, Kevin McCullum and James Irvine

Investigating concerns raised by Lac La Ronge Indian Band members about the safety of consuming traditionally harvested foods (PL) 150
Kelly Wells and Ryan Froess

Lower Churchill hydroelectric generation project: Dietary surveys and human biomonitoring to support the baseline human health risk assessment program (PL) 151
Rob Willis, Theresa Repaso-Subang, Jackie Wells, Peter Madden, Leah Fudge and Rosanne Williams

A community-based cumulative effects monitoring program for the Slave River, Northwest Territories, Canada (PL) 151
Paul Jones, Tim Jardine, Lalita Bharadwaj, Karl Lindenschmidt, Lorne Doig, Erin Kelly and Sarah Rosolen

Slave River Environmental Effects Monitoring Program (SWEEP) and benthic macroinvertebrates: A community-based approach to monitoring the long-term health of a large northern river (PL) 152
Lorne Doig, Jan Ciborowski, Michelle Dobrin, Edward Moes, Victor Mandeville, Frasier McTurk, Sarah Rosolen and Slave River and Delta Partnership

Predicting mercury distribution in the Slave River and Delta using geospatial modeling (PL) 153
Lorne Doig, Anna Meissner, Tim Jardine, Paul Jones, Lalita Bharadwaj and Karl-Erich Lindenschmidt

Author Index 154

Dr. Richard C. Playle Awards for Outstanding Theses in Ecotoxicology

The 2015 winner of the MSc thesis award was Laura Phalen, University of Prince Edward Island. No BSc Honours thesis award was awarded in 2015.

Rainbow trout (*Oncorhynchus mykiss*) thrombocytes and B cells show similar expression of cytochrome, AhR, and P53 mRNA following *in vivo* exposure to benzo[a]pyrene (PL)

Laura J. Phalen[1], Bernd Köllner[2], Natacha S. Hogan[3] and Michael van den Heuvel[1]
[1]University of Prince Edward Island, [2]Friedrich Loeffler Institute, [3]University of Saskatchewan

Polycyclic aromatic hydrocarbons (PAHs) are a group of compounds with immunotoxic and carcinogenic potential that may pose a threat to fish populations. The mechanism underlying immune toxicity of PAHs in fishes remains unclear. Some evidence supports the requirement for metabolism to more toxic metabolites. This study examined whether intraperitoneal exposure of rainbow trout (*Oncorhynchus mykiss*) to 100 $mg \cdot kg^{-1}$ benzo[a]pyrene (BaP) caused leukocyte mRNA expression changes in five cytochrome P450 (CYP) enzymes; to their transcription factor, AhR; or to an extrinsic pathway apoptosis checkpoint, p53. mRNA expression was analyzed in immunomagnetically isolated B cells and thrombocytes from blood, spleen, or head kidney in an effort to clarify the tissue- and cell-specific toxicity of BaP that was observed in an earlier study. Significant inductions above control levels were observed in CYP1A1 in liver, blood B cells, and blood thrombocytes; CYP1B1 in blood B cells, and blood thrombocytes; CYP1A3 in liver, blood and spleen B cells; and AhR in spleen thrombocytes. No significant changes were found in CYP1C1, CYP1C2, or p53. Increased mRNA expression was observed 14 days after exposure, indicating a prolonged physiological effect of a single BaP injection. Overall, our findings provide support for a complex mechanism of toxicity for chronic PAH exposure, and suggest that CYP expression profiles do not fully explain these effects.

Contaminants and Ecosystem Health

How much fish mortality are Environmental Effects Monitoring lethal fish population monitoring programs causing and is this a concern in small systems? (PL)

Cassandra Rees[1], Kelly Wells[1] and Jennifer Doucette[1]
[1]Canada North Environmental Services

Environment Canada's Metal Mining Environmental Effects Monitoring (EEM) program is designed to detect and measure changes in aquatic ecosystems downstream of treated effluent discharge locations. The EEM program objective is to ensure that fish and their environment are sufficiently protected by the *Metal Mining Effluent Regulations*. Biological monitoring studies are conducted to determine if mine effluent is having an effect on fish, fish habitat, and fisheries resources. Fish population surveys examine effect endpoints related to survival, energy use, and energy storage in preferably two sentinel fish species. Lethal sampling of adult fish in an area proximate to treated effluent discharge and a minimum of one reference area is the preferred study design in order to collect data on EEM-defined effect endpoints such as age, liver weight, and gonad weight. This study design requires lethal fish sampling that is conducted on the same populations every three years. A minimum of 20 individuals per sex from each sentinel species is required; however, many more are often sacrificed to collect sufficient numbers from both sexes or to improve statistical power. Commonly, more than 100 individuals per sentinel species are sacrificed from a single sampling area during one monitoring cycle, plus additional incidental mortality often occurs through by-catch. Although this might not have an effect in large systems, in small, isolated systems density-dependent effects are more likely. For example, fishing pressure is well known to alter size and age-structure of fish populations, two metrics that are examined for effects as part of the EEM program. Furthermore, there is less immigration in these systems to aid in recovery after fish are removed. We will provide statistics on the number of fish sacrificed by lethal EEM surveys and will present case studies from small watersheds where lethal fish sampling was likely the cause of reduced catch-per-unit-effort in subsequent monitoring years. Whether repeated lethal fish population surveys, akin to fishing, are causing significant effects on fish populations (particularly in EEM-defined effect endpoints) deserves greater attention. It is also important to thoroughly evaluate if the information gained by lethal fish sampling is meeting the objectives of the EEM program and whether non-lethal assessments could be more widely used as surrogates.

Strengthening environmental toxicology capacity in developing countries: Ghana case example (PL)

Nil Basu[1], Julius Fobil[2] and Thomas Robins[3]
[1]McGill University, [2]University of Ghana, [3]University of Michigan

The advancement of environmental toxicology programs in low- and middle-income countries is challenged owing to limited in-country resources and capacity. Here I detail three distinct, yet mutually reinforcing, North-South collaborative projects based in Ghana that are expected to lead to tangibly improving environmental toxicology-related research practice and training and to increasing scientific knowledge and technical ability in Ghana and the larger West Africa region. Program #1 (GEOHealth; 2012-present) has taken key steps in Ghana to strengthen degree-granting educational programs in public health, in-country research skills and projects, and partnerships across stakeholder groups (e.g., Ghana Health Service, civil societies, industries and trade unions, etc.). Program #2 (PARTNER; 2011-present) has provided a range of opportunities for junior- to mid-level Ghanaian researchers to better address in-country global health challenges. Program #3 (Integrated Assessment of artisanal and small-scale gold mining in Ghana; 2009-present) has brought together 50+ in-country stakeholders to co-develop policy/response options to informal gold mining, and is currently helping inform Ghana's National Action Plan towards the United Nations' Minamata Convention. The talk will briefly describe each of the efforts and focus on lessons learned (e.g., mentorship continuum from junior to senior and North-South; topics that lend themselves to multidisciplinary collaborations; employing regular and diverse communication; meeting regularly and building trust) in terms of strengthening capacity in low- and middle-income countries so as to possibly inform other regions.

Who and what reflects ecosystem health? A case study based on the oil sands (PL)

Judit Smits[1]
[1]University of Calgary

This presentation will explore what is meant by the extremely popular idea of Ecosystem Health. I will discuss how this amorphous concept may be evaluated using examples from research that has occurred in and around the oil sands for the past one and a half decades. The first toxicology or contaminant research that took precedence was to identify the potentially offending substances that are being exposed, concentrated, or created in the various processes involved in extracting oil from these deposits. First the oil-impregnated sand must be mined, and then the heavy, molasses-like bitumen must be extracted from the sand using alkali steam. Then the non-commercial products are contained in tailings ponds, percolated through wetlands, or released to the air as volatile or particulate matter. Here there have been numerous monitoring programs carried out for decades, as a means of measuring how much or what compounds are being found where. Arguably, these results from the massive, expensive, and extensive monitoring efforts are of interest primarily because of the potential for sustained, detrimental, biological effects. My multi-species approach to understanding the health of ecosystems in the oil sands region will be incorporated into this forum in which I will present a summary of the biological studies that have been carried out, focussed primarily on vertebrates. The presentation will elaborate the biological components of interest (e.g., invertebrates, plants, fish, birds, mammals) and how they can be assessed for the features that contribute to a resilient ecosystem. But I will also acknowledge the valuable contributions of plant, microbial and hydrological research to date that have formed components of the ecosystem health related studies to date, in an effort to direct and encourage sustainable development of natural resources on which we all depend.

Health status of fishes from the Athabasca and Slave River system, northern Canada (PL)

Paul Jones[1], Allison Hill[1], Brett Tendler[1], Ehimai Ohiozebau[1], Erin Kelly[2] and John Giesy[1]

[1]University of Saskatchewan, [2]Government of the Northwest Territories

The Slave River Delta is one of Canada's largest freshwater delta systems. The Slave River is fed by the Athabasca and Peace rivers, which flow through Northern Alberta. Increased industrial activity in the Peace/Athabasca River basin, particularly oil sands development, has been a source of ongoing concern to First Nation and Métis communities as the Slave River and Delta are part of their traditional way of life. Concerns about the health of fish in the system are related to potential adverse impacts of chemical contaminants from upstream industrial activities on the health of fish and the humans that consume them. Local concerns have been heightened by the occurrence of abnormalities or lesion on some fish collected from the system. This study was designed to perform a systematic assessment of the occurrence of lesions in the system and to gather tissues for supporting health information. Fish were collected from the river system with the assistance of local fishers to ensure that species of concern to local communities were collected from locations most commonly used for harvesting. Fish were sampled between 2011 and 2014 to assess temporal variation. Fish were assessed by a detailed external and internal health assessment compatible with Canadian Environmental Effects Monitoring standards. Tissues were collected and archived for future analysis. All dissections were photographically documented. Lesions and other abnormalities were encountered in the sampled fish but their distribution was relatively uniform. Morphometric data demonstrated relatively consistent fish health in both the Athabasca and Slave rivers, and analysis of condition factor and somatic indices did not demonstrate any consistent impacts along the river system. Overall the health of fish does not appear to be impacted at current levels of oil sands development. However, the potential for cumulative impacts from oil sands operations in concert with other stressors suggests the need for sustained monitoring.

Short-term recovery of benthic macro invertebrate assemblages from a Jet A-1 fuel spill in Lemon Creek, British Columbia (PL)

Cory Bettles[1], Cliff Robinson[1] and Jason Baird[1]
[1]SNC Lavalin Inc.

A benthic macro invertebrate assessment, adapted from guidelines for sampling benthic invertebrates in British Columbia streams and the Canadian Aquatic Biomonitoring Network (CABIN), was conducted on Lemon Creek British Columbia to assess the impact on, and recovery of, the invertebrate assemblage that was exposed to a Jet A-1 fuel spill in August 2013. Two benthic invertebrate references stations located 0.3-1.0 km upstream of the fuel spill and four exposure stations located 1-4 km downstream of the spill were sampled using kick nets in October 2013 and spring and fall 2014. A reference condition approach (CABIN) was used to determine if invertebrate assemblages, biodiversity metrics, and habitat data at the sites were similar to CABIN-selected reference sites, and if not, the degree to which they were impaired. A secondary non-parametric multivariate analysis of invertebrate assemblage structure was used to evaluate differences between reference and exposure sites over time. Finally, a simple gradient analysis of assemblages and metrics from upstream to downstream sites was used to assess the degree of impairment and recovery of the benthic invertebrate assemblage over time. Recovery was defined as meeting biometric endpoints, including invertebrate taxa abundance and richness (both total and Ephemeroptera- Plecoptera- Tricoptera (EPT)), as well as the percentage of EPT taxonomic families that are considered pollution-sensitive. These metrics initially exhibited a decrease in abundance levels at exposure sites but resembled reference sites one year later. Additionally, the percent of Oligochaetes and Chironomids, two taxa families that are considered pollution-tolerant, while initially high at exposure sites in October 2013, decreased to levels similar to reference sites by October 2014. Overall, endpoint metrics of species abundance, richness, percent EPT, and percent Oligochaete and Chironomids, and the increase in the River Invertebrate Prediction And Classification System (RIVPACS) ratio indicate that exposure sites are in good condition as of October 2014 and that the benthic invertebrate community has recovered to reference site levels. CABIN BEnthic Assessment of SedimenT (BEAST) analysis results confirmed that all exposure sites on Lemon Creek were only mildly divergent by October 2014, and hence were considered similar in composition to CABIN-selected reference sites. Results from gradient analyses indicated a strong downstream response in endpoint metrics to the fuel spill in October 2013 that subsequently subsided by October 2014. In summary, based on a weight-of-evidence approach, a Jet A-1 fuel spill in Lemon Creek initially impacted the benthic invertebrate community at sites downstream of the spill but the assemblage showed recovery by spring 2014 and was indistinguishable from reference conditions by October 2014.

Effects of agricultural intensity and pesticide use on abundance of six aerial insectivorous bird species over 30 years in Canada's Prairie Pothole Region (PL)

Nicole Michel[1], Robert Clark[2], Keith Hobson[2] and Christy Morrissey[1]
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Aerial insectivorous birds are undergoing severe population declines across North America. Analyses of North American Breeding Bird Survey (BBS) data revealed negative population trends in 20 of 33 species since 1966, with aerial insectivores experiencing the most severe decline of any avian guild in Canada. Aerial insectivorous birds face a variety of threats such as pesticides, habitat loss (including conversion to high-intensity agriculture), and climate change. However, the relative effects of these potential drivers of population trend remain unknown. Here, we evaluate the relative effects of pesticide use, agricultural intensity, land cover, climate, and weather on a suite of six aerial insectivorous bird species: purple martin (*Progne subis*), tree swallow (*Tachycineta bicolor*), northern rough-winged swallow (*Stelgidopteryx serripennis*), bank swallow (*Riparia riparia*), cliff swallow (*Petrochelidon pyrrhonota*), and barn swallow (*Hirundo rustica*). We used boosted regression trees, a machine-learning technique, to assess the ability of spatiotemporally dynamic variables measured on three nested scales to predict aerial insectivorous bird abundance. Our analyses encompassed 117 BBS routes across the Prairie Pothole region of Alberta and Saskatchewan over a 30-year period. Pesticide use explained 2% (northern rough-winged swallow) to 11% (bank swallow) of the variation in abundance, while agricultural intensity (e.g., proportion cropland, number of cattle) explained up to 93% (northern rough-winged swallow). While pesticide use was not the primary driver of population variation for any species in this analysis, the coarse scale of available data may have limited our ability to detect effects. Moreover, the effects of agricultural intensity on insectivorous bird abundance may be mediated by direct and indirect effects of pesticides. Though further studies with finer-scale data are encouraged, our findings have important implications for aerial insectivorous bird conservation and management in Canada's Prairie Pothole Region.

Spatio-temporal variation of metal concentrations in wetlands and amphibians from the oil sands region of northern Alberta, Canada (PL)

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Among the pollutants potentially released during oil sands mining and upgrading, metals are of concern because of their potential for toxicity, bioaccumulation and in some cases, biomagnification. We investigated spatio-temporal patterns of five metals of concern in wetlands and amphibians as part of a larger, multi-year project designed to address knowledge gaps and evaluate wetland and wildlife health in the oil sands region. Total concentrations of mercury (Hg), arsenic (As), cadmium (Cd), lead (Pb) and selenium (Se) were measured in water and wood frogs (*Lithobates sylvaticus*) collected from 13

wetlands multiple times between May-September, from 2012 to 2014. Data on general water chemistry and amphibian health were also collected. Wood frogs were chosen as a focal species because they are abundant across the study region and their life cycle includes both aquatic and terrestrial stages, allowing us to examine differences among stages. The wetlands are situated at varying distance from oil sands industrial activities. Across all sites, levels of Cd and Se in water were very low, and more than 70% of samples had levels below their respective detection limits (dl): Cd: <dl-0.05 $\mu g \cdot L^{-1}$; Se: <dl-0.39 $\mu g \cdot L^{-1}$. Median levels of Hg, As and Pb in water were 2.08 (0.34-21.3) $ng \cdot L^{-1}$, 0.72 (<dl-22.2) $\mu g \cdot L^{-1}$ and 0.05 (<dl-0.66) $\mu g \cdot L^{-1}$, respectively, and varied among sites. Concentrations were well below guidelines published by the Canadian Council of Ministers of the Environment (CCME). Linear mixed models indicated that levels of metals in breeding pond water were not associated with distance from upgraders or with time (within or across years), but were related to the concentrations of other metals present in water. In amphibian tissues, median levels of Cd and Pb were very low, and more than 50% of samples had levels below their respective detection limits (Cd: 0.12 (<dl-0.46), Pb: <dl (<dl-2.79) $\mu g \cdot g^{-1}$ dry weight (dw)). Median Hg, As and Se levels in tissues were 0.09 (0.02-0.41), 0.21 (<dl-12.79), and 0.65 (<dl-1.59) $\mu g \cdot g^{-1}$ (dw), respectively. Concentrations of Hg and Se varied among sites. Hg, Cd and Se did not vary among wood frog life stages, whereas As and Pb were significantly higher in tadpoles compared to recent metamorphs and adults. Linear mixed models indicated that, in general, variation in tissue metal concentrations was not related to distance from upgraders nor to time (within or across years), but was related to other metals present in water and tissues, and in a few cases, linked with amphibian life stages. Levels of methylmercury in amphibian tissue as well as other indicators of amphibian health such as prevalence of infectious diseases and measures of stress are being examined. Results from our study will inform long-term monitoring plans in the oil sands region, particularly for monitoring the health of wetland ecosystems in the region.

Adaptive management in Canada's Northwest Territories: A case study of the application of prediction intervals to quantify natural variability (PL)

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Aquatic environmental monitoring studies typically quantify biological change by performing statistical tests on relevant endpoints from an exposure and a reference area. Recent guidance from the Northwest Territories (NWT) Water Boards has encouraged incorporation of an adaptive management approach towards aquatic effects monitoring programs in the NWT. The successful implementation of adaptive management requires the inclusion of biologically and ecologically meaningful endpoints to serve as action level triggers, but also an understanding of the magnitude of change in these endpoints that is indicative of natural variability versus changes that are due to anthropogenic sources. A temporal reference normal-range decision matrix was implemented to trigger an action level for thallium and cesium in fish tissue. The incorporation of normal range into the decision matrix strengthened the confidence with which the action level was triggered, identifying the need for further investigation into the cause of an increase in thallium and cesium concentrations in fish tissue. The results of this investigation will be discussed.

Contamination and multi-decadal decay of reservoir-liberated mercury in a downstream fishery: Effects of fish trophic level, size and age (PL)

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Aquatic mercury (Hg) can be biomethylated into a potent neurotoxin, MeHg. Hydroelectric reservoir construction is frequently responsible for bioaccumulation of MeHg within endemic biota. Mercury concentration ([Hg]) in fish impounded in artificial reservoirs and affected downstream sites can exceed this limit and take decades to decay below the 0.5 $mg{\cdot}kg^{-1}$ consumption guideline. The present study examines the characteristics of fish affecting mercury decay from a hydroelectric reservoir created in 1963 and an affected downstream fishery in the Saskatchewan River Delta. Rates of decay in Tobin Lake (reservoir) and Cumberland Lake (delta) were analyzed using exponential regression. Northern pike (*Esox lucius*), sauger (*Sander canadensis*), goldeye (*Hiodon alosoides*), and walleye (*Sander vitreus*) all showed significant decay rates between the 1970s and present (Tobin: $p<0.05$ for all regressions; Cumberland: $p<0.01$ for all regressions). In *S. vitreus*, contemporary tissue [Hg] was strongly correlated with length, age, and reservoir proximity. Stable nitrogen isotopes were used to assess correlations between Hg and trophic levels, but explained little additional variation beyond that accounted by length and age. Our results suggest that contemporary fish [Hg] have decayed below consumption guidelines.

Recovery of a mining-influenced stream ecosystem (PL)

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The environmental science literature is replete with case studies of stream ecosystems damaged by metals pollution, but fewer case studies are available with long-term data on the recovery from effects of such pollution. Panther Creek, a tributary of the Salmon River in central Idaho, U.S., is one such case with a 30-plus year period of record from about 1980 through 2014. Panther Creek suffered intensive damage from large-scale mining and milling operations in the Blackbird Mining District, primarily from the late 1940s to 1967. The mine drained to two tributaries of Panther Creek, Blackbird

Creek and Big Deer Creek, contaminating the streams with copper (Cu), arsenic (As), and cobalt (Co), as well as altering the physical channel. From the 1960s through 1980s, no fish and few aquatic insects could be found in mine-affected sections of Panther Creek. By the early 1990s, Cu concentrations had attenuated to the point that some natural production of rainbow trout *(Oncorhynchus mykiss)* occurred. However, Chinook salmon *(Oncorhynchus tshawytscha* and resident fish other than rainbow trout remained rare. Species richness of aquatic insects was about half that of reference sites, and mayflies were absent or rare. Concerted efforts to reduce mine contamination began in 1995, and by 2002 Cu and Co levels had been reduced by about 90%. As of 2014, resident fish populations in mining-influenced reaches appeared to have recovered, relative to reference reaches. Rainbow trout were early colonizers, quickly expanding their range as areas became habitable. Sculpin appeared slower to disperse and colonize; but they appeared to fully recover about four years after their first detections. Macroinvertebrate richness in Panther Creek sites downstream of Blackbird and Big Deer Creeks appears to have plateaued at about 70 to 90% of reference. Total abundances of mayflies in locations downstream of mine influences are as high as or higher than at reference locations. However, mayfly diversity remains lower than reference, and Heptageniids and Ephemerellids remain rare. These patterns raise questions of what it means for an ecosystem to be "recovered". For example, if benthic macroinvertebrates provide a sufficient food base for fish populations, is the ecosystem sufficiently recovered? Or would all major ecosystem components need to be similar to reference?

Mercury biomagnification through lake food webs with different top predator fishes (PL)

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Mercury (Hg) biomagnification through aquatic food webs is quantified using regression slopes of biotic log-Hg concentrations versus nitrogen isotope values ($\delta^{15}N$; expressed as trophic magnification slopes—TMS). TMSs are related to some physical and chemical characteristics of ecosystems on both regional and global scales, but their influence can vary across regions or scales. Principal component analysis (PCA) in combination with multiple regression modeling assesses the physico-chemical variables that are related to Hg TMS values among regional ecosystems, but this approach has not been tested on a larger group of food webs with diverse top predator fishes. In this study, we determined Hg TMS values in five lake food webs supporting brook trout (*Salvelinus fontinalis*) in New Brunswick through collections and analysis of littoral and pelagic invertebrates and fishes for MeHg (or total Hg for fish) and $\delta^{15}N$. TMS values ranged from 0.128-0.207 across these lakes. We then combined results from the brook trout lakes with comparable data from food webs supporting other fishes across temperate and Arctic regions of Canada (Arctic char (*S. alpinus*), lake trout (*S. namaycush*), and yellow perch (*Perca flavescens*)) to determine whether lake physicochemical characteristics were related to Hg TMS values across a larger number of systems (n=36 lakes). A combination of longitude, lake depth, and cation concentrations (grouped by PCA) positively predicted the TMS across lakes (multiple regression, R^2Adj=0.153, p=0.025). However, when food webs supporting different top predator fishes were examined separately, the direction of these relationships differed (e.g., R=-0.89 and p=0.008 for yellow perch food webs, R=0.62 and p=0.033 for lake trout food webs). In conclusion, these analyses suggest that Hg biomagnification was affected by system characteristics but that their degree of influence was not necessarily broadly predictable.

Contaminants in the Arctic terrestrial ecosystem (PL)

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Contaminants in the Arctic terrestrial ecosystem are generally low, particularly when compared with the marine environment, and are not generally of toxicological concern for wildlife or consumers of wildlife. There are, however, several exceptions to this. Caribou *(Rangifer tarandus)* liver was found to have the highest concentration of perfluorooctane sulfonate (PFOS) and perfluorocarboxylates (PFCAs) among traditional food collected from Nunavut in 1997 and 1999. Polybrominated diphenyl ethers (PBDEs) in caribou may also represent an important dietary source of contaminants to humans. Renal and hepatic mercury in caribou may cause some concern for human health depending on the quantity of organs consumed, while renal and hepatic cadmium in Arctic moose and caribou have prompted health advisories in Yukon and NWT. When considering wildlife, it is often difficult to determine if and how a particular contaminant impacts an individual animal, it often being necessary to extrapolate from toxicity data for domestic animals. It is even more difficult to determine the effect of contaminants on wildlife populations. However, a recent study on the Akia-Maniitsoq and Kangerlussuaq-Sisimiut caribou herds from West Greenland suggests that hepatic mercury concentrations above 0.5 $\mu g \cdot g^{-1}$ (dry weight) may negatively affect fertility in caribou cows. In addition, hepatic mercury levels were negatively correlated with cow body weight, suggesting an adverse effect on body condition. This could be an indication of a sub-acute effect on individual animals, but a significant effect on the population.

Bioaccumulation of pharmaceuticals and personal care products in freshwater mussels *(Lasmigona costata)* in a river receiving wastewater effluent (PL)

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Freshwater mussels are frequently found in rivers receiving effluent from municipal wastewater treatment plants (WWTP), and there is strong evidence that poor water quality is deleterious to mussel populations. Studies have shown that wild freshwater mussels downstream of WWTPs in the Grand River, Ontario, exhibited oxidative stress, reduced condition and shortened lifespans compared to mussels living upstream. In addition, exposure to WWTP effluent has been shown to induce oxidative stress in field-deployed mussels. WWTPs are among the main sources of pharmaceuticals and personal care products (PPCPs) in surface waters. We monitored PPCPs in wild and caged mussels both upstream and downstream of a major WWTP on the Grand River, as well as in water, in 2010 and 2012. Our objectives were to characterize the seasonal changes in PPCP concentrations in water, to calculate the bioaccumulation factors (BAFs) of PPCPs in mussels, and to determine the chemical and physical properties of PPCPs driving the bioaccumulation. Aqueous concentrations were highest in the summer or early fall, which corresponded to low river flow. Forty-three PPCPs from a variety of pharmaceutical classes were detected in mussel tissues, including stimulants, anti-inflammatory drugs, anti-bacterial agents, antibiotics, antidepressants, antihistamines, progestins, and illicit drugs including cocaine and amphetamines. The BAFs ranged from 0.66 for metformin (antidiabetic drug) to 32,022 for sertraline (antidepressant). BAFs were predictable from log K_{OC}, log K_{OW}, and sediment fugacity ratio ($R^2X=0.70$) in a manner consistent with expectations based upon BCF models and the physical and chemical characteristics of each compound. Although we also used laboratory exposures to assess the toxicity of the most prevalent PPCPs found in mussel tissues, given the complex nature of WWTP effluent, the contribution of PPCPs to the cumulative negative effects of urban inputs on freshwater mussels is not yet known.

Synthesizing spatiotemporal patterns and ecological drivers of neonicotinoid fate in Canadian Prairie Pothole wetlands (PL)

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Since the Green Revolution of the 1960s, modern agricultural practices have relied heavily on mechanization, large-scale production of monoculture crops, and the use of numerous chemical inputs including fertilizers, herbicides and insecticides. However, the advent of the neonicotinoid seed treatments, registered for a large number of crops, has promoted pervasive use across large spatial extents. This may further be leading to degradation of regional wetland water quality and associated ecosystem stability particularly in areas of high wetland density and agricultural intensity such as the Canadian Prairies. Between 2012 and 2014, we completed a series of field sampling studies of over 400 Prairie wetlands across multiple geographic areas of Saskatchewan. Our results indicate that detection of neonicotinoids in study wetlands varied between 16% (fall 2012) and 91% (spring 2013). With minor differences among crop types, peak summed neonicotinoid concentrations consistently occurred during summer sampling (~6 weeks post-seeding): 3,110 ng·L^{-1}(2012); 595 ng·L^{-1}(2013) and

2,092 ng·L^{-1}(2014). Because neonicotinoid concentrations varied widely between wetlands that were ecologically and physically similar, we conducted detailed wetland assessments to document wetland and landscape features which could potentially affect their fate in Prairie ponds. Our exploratory analysis identified that plant composition, not density, is a key driver of both neonicotinoid detection and concentration in Prairie wetlands during summer. In addition, neonicotinoids were consistently detected in a high percentage of wetlands in spring, after ice-off but before seeding had occurred, for reasons that we further explored. In spring 2014, we investigated whether snow meltwater, particulate matter, top- (15 cm) or bottom-layer (15 cm) snow were sources of spring neonicotinoid contamination to wetlands. Meltwater was identified as the primary contributor of neonicotinoids to spring wetland water (R^2=0.42). In both spring and summer, temporary wetlands were associated with higher neonicotinoid concentrations more than any other wetland class, which may have important implications for wetland biota. The outcomes of this full study will lead to better understanding of the environmental fate of neonicotinoids in aquatic environments such as agricultural wetlands and offer insight into mitigation strategies to improve wetland ecosystem health.

Overview of DFO's National Contaminants Advisory Group: Assessing biological effects of contaminants on aquatic ecosystems (PO)

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As a science-based federal government department, Fisheries and Oceans Canada (DFO) requires scientific evidence to facilitate the sound management of Canada's fisheries, and to advance sustainable aquatic ecosystems while fostering economic prosperity across maritime sectors and fisheries. The National Contaminants Advisory Group (NCAG) was formed in January 2013 to provide scientific information and advice to DFO on priority contaminants issues. The main functions of the group are to facilitate research projects through external researchers, to synthesize results and to develop science advice in support of DFO decision-making. Current priority research themes are: (1) oil and gas, (2) pesticides, (3) aquaculture therapeutants, and (4) contaminants of emerging concern. NCAG administers research funds through a variety of mechanisms, including two Calls for Proposals that have resulted in funding for multiyear research projects at Canadian universities. Completed and ongoing NCAG science advisory activities are summarized, and future directions are addressed.

Analysis of hydrological parameter effects on organohalogenated micropollutant (polybrominated diphenyl ethers and polychlorinated biphenyls) behaviour: Case study of the Seine River in France (PO)

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Polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) are two organohalogenated micropollutants that have been widely used in past decades in many industrial applications; however, there is not much information related to their dynamic in water resources reservoirs. As first attempt, the sequential dynamics of PBDEs and PCBs were investigated during a flood cycle of six months' duration in a heavily urbanized urban stretch of the Seine River (Paris, France). The results show that pollutant concentrations ranged from 0.2 to 3.8 $ng{\cdot}L^{-1}$ (median 1.1) and 0.4 to 3.6 $ng{\cdot}L^{-1}$ (median 1.1) for Σ tri-deca BDEs and Σ 7 PCBs, respectively. Furthermore, the partitioning between the particulate and dissolved phases was investigated for both PBDEs and PCBs. Due to their low water solubility, PBDEs were mainly (more than 70%) bound to particles. However, only 54% of Σ 7PCBs occurred in the particulate phase, and their partitioning was influenced by the degree of chlorination. During the sampling period, PBDE and PCB fluxes were quite similar and ranged from 3 to 128 $g{\cdot}day^{-1}$ and from 6 to 125 $g{\cdot}day^{-1}$, respectively. The annual mass flow of PBDEs and PCBs was estimated to 10 kg for both. Dissimilar to PCBs and BDE-209, the temporal evolution of Σ tri-hepta BDEs was related to particulate organic matter content, which is controlled by river flow variations. This suggests that they might exhibit different sources or behaviour in the Seine River.

Factors affecting amphipod communities in estuaries of the Southern Gulf of St. Lawrence: Are contaminants affecting crustacean survival? (PO)

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Increased nitrogen loading into estuaries feeding the Southern Gulf of St Lawrence, here defined as estuaries entering the Northumberland Strait from New Brunswick (NB) and Nova Scotia (NS) and all of Prince Edward Island (PEI), has increased the abundance of macro- and microalgae in estuarine systems. Land-use in PEI is primarily agricultural, with the majority of the acreage in potato production. Consequently, PEI is the primary source of nutrients to the Northumberland Strait and may also be responsible for greater sediment and contaminant deposition than NB or NS. Effects of eutrophication are well understood, with an increase in abundance of some species, in the estuary amphipods (Crustacea: Amphipoda), and a decrease in dissolved oxygen. However, invertebrate surveys conducted in thirteen Southern Gulf estuaries in macroalgae (*Ulva* spp.) and eelgrass (*Zostera marina*) habitats in June 2013, before summer hypoxia occurs, do not fully support this hypothesis. In some estuaries with high nitrogen loading the expected increase in abundance of amphipod species (*Gammarus oceanicus*, *G. lawrencianus* and *G. mucronatus*) was observed but in other estuaries with similar nitrogen loading it was not. This variability suggests other factors are influencing population numbers and community composition in the upper estuary. Coupled with upper estuary issues there has been a marked decline in lobster (*Homarus americanus*) landings and juvenile recruitment in the Northumberland Strait since the 1990s. We hypothesize that sediment and pesticide runoff from agricultural fields may account for these differences and that upper estuary crustacean populations (i.e., amphipods) may indicate effects on crustacean species farther out on the coast. To test these effects, acute bioassays will be set up to test the relative sensitivities of the collected species to commonly used pesticides on PEI (e.g., chlorothalonil, linuron, cypermethrin, etc.). Sediment bioassays will be performed to test the relative sensitivities and vital rates (growth, fecundity, mortality) among species in affected sediments. Finally, dietary comparisons between amphipods will be carried out using Next Generation Sequencing to examine if certain food sources are being adversely affected by fungicides, herbicides, or even insecticides and thus limiting food availability. These experiments and surveys will either support the hypothesis that contaminants are influencing species differences or highlight an unmeasured natural factor causing the variability. Results may inform changes in management for fisheries in the region.

General Aquatic Toxicology

Can a bioaccumulation saturation model derived for the benthic invertebrate *Hyalella azteca* be a good predictor of uranium toxicity under natural conditions? (PL)

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A uranium bioaccumulation saturation model is a good tool for risk assessors and regulators to reliably predict uranium toxicity. This model, which is based on a critical burden concentration, takes into consideration toxicity modifying factors such as pH, alkalinity, hardness and dissolved organic carbon (DOC) in the water that influence uranium speciation, bioavailability, bioaccumulation and toxicity to organisms. In this study, critical body concentrations of uranium in *Hyalella azteca* exposed to different overlying water chemistries and different uranium concentrations in the sediment are found to be a more accurate predictor of uranium toxicity than concentrations in the water and sediment. The water rather than sediment phase is found to be the main route of uranium uptake by this organism. A mechanistic bioaccumulation saturation model is shown to be better at fitting the data than an allometric equation. The hydrogen ion is found to have a major effect on uranium bioaccumulation, while the calcium ion is found only to influence uranium speciation. To validate the uranium bioaccumulation saturation model to predict uranium toxicity based on a critical body concentration of 50% survival (LCB_{50}) in the field, field data compiled from the literature were used to test the model. The observed versus predicted ratios (n=9) based on LCB_{50}s were between 0.3 and 4.6. Considering that our study did not evaluate the effect of DOC on uranium bioaccumulation, the over prediction of uranium toxicity to *H. azteca* (n=1) may have been the result of elevated DOC concentrations in the water that have been found in the literature to mitigate uranium toxicity. In cases where the model under predicted toxicity (n=5), uranium concentrations in the water were close to background levels (n=3) or outside the model pH range (6.9-8.0). In general, the model is in good agreement with the field data in cases (n=3) where uranium water concentrations were above background and within the range of pH values used to derive the model. In these cases, the observed versus predicted LCB_{50} ratios were 0.9 to 1.0. To make the model more applicable under different uranium water chemistries, pH values and natural conditions near uranium mines and mills, field experiments with different uranium water chemistries and concentrations are required to refine and test the bioaccumulation saturation model.

Chronic nickel and copper toxicity in the cryptic *Hyalella azteca* species complex (PL)

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The amphipod crustacean *Hyalella azteca* was thought to be a single species, but recent molecular studies have indicated that it is a large cryptic species complex. Previous work has indicated that the *H. azteca* complex contains up to 85 genetically divergent lineages. *H. azteca* has been frequently used in toxicity tests due to its sensitivity to contaminants, but past studies have examined the effects of contaminant toxicity with the assumption that *H. azteca* is a single species rather than a large cryptic species complex. Recently, a research group determined that two genetically characterized laboratory lineages (Clade 1 and 8) display different nickel and copper sensitivity in acute toxicity tests when assessing lethal concentrations, but not lethal body concentrations. In this study, *Hyalella* Clades 1 and 8 were subject to 28-day nickel and copper toxicity tests, and their survival and growth responses were compared. The estimated nickel and copper LC_{50}s for Clade 8 were 1.74 and 1.42 times greater than those of Clade 1, respectively. These results complement an earlier study in that at least two lineages within the *H. azteca* cryptic species complex display different sensitivities to nickel and copper in both acute and chronic toxicity tests. Therefore, caution should be used when conducting toxicity tests with laboratory cultures that have not been genetically characterized as the results obtained may not be comparable.

Effects of mercury on the health of wild fish populations in Kejimkujik National Park, Nova Scotia, Canada (PL)

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Mercury (Hg) is a global pollutant that is released into the atmosphere through industrial activities such as the burning of fossil fuels, and it can be deposited into lakes far from its point source. In these lakes Hg is transformed into the organic contaminant methyl Hg (MeHg), which is known to biomagnify through the food web and negatively impact the physiology, behaviour, and endocrine and immune functions of fishes and fish-eating wildlife. This study examined whether the overall health of wild fishes at Kejimkujik National Park (KNP), Nova Scotia, Canada, known as a biological Hg "hotspot", was negatively affected by Hg exposure. A previous study on yellow perch (*Perca flavescens*) from lakes in KNP found that fish with higher total Hg (THg, representative of MeHg) had greater damage in the liver, spleen, and kidney, as evidenced by increased macrophage aggregates. Herein, we examined whether this trend was also found for other fishes in these lakes. Brown bullhead (*Ameiurus nebulosus*), banded killifish (*Fundulus diaphanus*), and golden shiner (*Notemigonus crysoleucas*) were collected in September 2013 and September 2014 from six lakes (10 males and 10 females per lake) in KNP representing a range in fish Hg concentrations. Fish were weighed and measured,

liver was weighed, liver, kidney and spleen were removed for histological analysis, and muscle was taken for THg analysis. Muscle THg of lakes ranged from (mean ± SD) 0.20 ± 0.05 to 0.50 ± 0.16 $\mu g \cdot g^{-1}$ wet weight (ww) in brown bullhead, 0.12 ± 0.04 to 0.55 ± 0.18 $\mu g \cdot g^{-1}$ ww in golden shiner and 0.17 ± 0.05 to 0.39 ± 0.10 $\mu g \cdot g^{-1}$ ww in banded killifish among lakes. Liver somatic index (LSI) was significantly lower in male and female brown bullhead from the higher Hg lakes, but no differences in condition between lakes were observed for this species. In banded killifish, there were significant differences in LSI and condition between lakes, but these were not related to mean Hg concentrations. Condition of golden shiner males and females was lowest in high Hg lakes, and LSI was significantly higher in the lowest Hg lake. All species showed evidence of greater tissue damage related to increasing Hg. Male and female brown bullhead from high Hg lakes had higher percent cover of MAs in the spleen, liver and kidney. Male and female golden shiner and banded killifish from high Hg lakes had higher percent cover of MAs in the spleen and liver. These findings suggest that the health of other wild fish species in KNP is also being affected by Hg, and that Hg concentrations similar to or greater than those found in the fish from KNP are of concern for the health of other populations of wild fishes.

Developing the brook stickleback (*Culaea inconstans*) as a novel bioindicator for exposure to endocrine disrupting compounds using (anti-)androgen and estrogen responsive biomarkers (PL)

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Small-bodied fish species are regularly used in both regulatory testing and ecological monitoring for endocrine disrupting compounds (EDC). However, each species exhibits specific strengths and weaknesses relative to EDC testing and screening. Most notably, freshwater species currently used in EDC studies lack sensitive and quantifiable biomarkers for (anti-)androgenic compounds. The brook stickleback (*Culaea inconstans*) is a small fish that inhabits freshwater environments throughout North America. Male brook stickleback produce a unique androgen-regulated glycoprotein in the kidney called spiggin. In other species of stickleback, induction of this protein in females has been used as a biomarker of exposure to exogenous androgens but it has never been characterised in brook stickleback. Therefore, our objective here was to develop a bioassay to measure estrogenic and (anti)androgenic responses in brook stickleback and to determine the response and sensitivity of various traditional and novel biomarkers of exposure. We first designed qPCR primers and optimised an assay to measure spiggin and vitellogenin transcript levels in kidney and liver tissue, respectively. Basal spiggin and vitellogenin transcripts were differentially expressed in mature wild-caught male and female brook stickleback. To determine the responsiveness and sensitivity of brook stickleback to EDCs, fish were exposed in-lab to a model androgen (17alpha-methyltestosterone, MT) or estrogen (17alpha-ethinylestradiol, EE2) at 1, 10 or 100 $ng \cdot L^{-1}$ for 21 days (sampled at 7 and 21 days) under a semi-static renewal system. In a third experiment, female fish were co-exposed to MT at 500 ng·L^{-1} and an anti-androgen (flutamide) at 25, 150 and 250 µg·L^{-1} for 14 days (sampled at 4 and 14 days) to validate this bioassay for the evaluation of anti-androgens. Exposure to MT and EE2 induced spiggin and vitellogenin transcripts in female kidneys and male livers, respectively. EE2 also significantly increased the hepatosomatic index in females after 7 days and in both sexes after 21 days. Histological analysis of the kidneys of MT-exposed females showed an increase in the kidney epithelium cell height after 7 days. In females, MT induced spiggin transcript levels and increased the nephrosomatic index while exposure to flutamide resulted in a dose dependent reduction of spiggin levels and the nephrosomatic index. This work is the first to evaluate molecular and apical changes in the brook stickleback in response to hormone exposure. Based on the sensitivity of these endpoints, we feel that the brook stickleback is a promising new freshwater fish model for EDC evaluation and a potential bioindicator for EDCs in freshwater environments of North America.

Acute cardiac effects of sublethal injections of benzo[a]pyrene and 5-azacytidine in juvenile rainbow trout (*Oncorrhyncus mykiss*) (PL)

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Benzo[a]pyrene (BaP) is a ubiquitous environmental contaminant that has recently been shown to exert acute cardiotoxicity in fish. BaP is rapidly metabolized, but has been reported to induce persistent effects via epigenetic modifications such as altered DNA methylation. 5-Azacytidine (5AZA) is known to inhibit DNA methylation and thus was used in this study to determine if this epigenetic mechanism plays any role in acute BaP cardiac toxicity in fish. Juvenile rainbow trout (*Oncorrhyncus mykiss*) were injected once daily for 2 consecutive days with control vehicle or BaP (0.1 and 1 mg·kg^{-1}) with or without 5AZA (2 mg·kg^{-1}) in a 2x2x3 factorial study design, then cardiac ultrasound was performed, followed by fish euthanasia on days 4 or 7 (n=10 fish/group/time). Liver, heart and red muscle were collected for analysis of glycogen and triglycerides (TG) content. Heart rate increased and duration of blood flow through the atrioventricular (AV) and ventriculobulbar (VB) valves and caudal aorta decreased with 5AZA alone at Day 4. Velocity of blood flow through AV valve during atrial diastole decreased with high BaP alone. When 5AZA was administered with BaP, all values were similar to control. All cardiac function values in all groups returned to control levels at Day 7. Thus, no evidence for persistence or potentiation (i.e., epigenetic mechanisms playing a role in acute cardiotoxicity) was found. In contrast, high BaP alone resulted in increased of TG content in heart while high BaP with 5AZA induced an increase in TG content in liver and muscle at Day 4. At Day 7, all TG values had returned to control levels; therefore, no evidence of persistence of effects was noted for this end-point. Lastly, BaP alone and 5AZA alone induced persistent depletion of glycogen storages in muscle and liver, respectively. An interaction between BaP and 5AZA resulted in persistently increased glycogen storages in the heart, but depletion of glycogen in the muscle only at Day 7. Taken together, trout underwent a shift towards more aerobic metabolism as evidenced by the reduced glycogen, but increased TG stores in liver and muscle after exposure to BaP, with DNA methylation playing a role in this response. This greater reliance on glycogen as an energy source may pose a threat to the survival of the trout given that utilization of TG is far more metabolically advantageous for long-distance swimming.

Sublethal effects of polymer-coated titanium dioxide nanoparticles and ultraviolet light co-exposure in developing zebrafish (*Danio rerio*) (PL)

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Nanoparticles (NPs) are used in many agricultural applications, from micronutrients for crops to carriers for pesticides. Most studies examine NP toxicity under fluorescent lamps that emit no ultraviolet (UV) light, yet certain NPs such as titanium dioxide (TiO2) absorb photons and generate electron-hole pairs that react with water and oxygen in the environment to produce reactive oxygen species. We tested aqueous polymer-coated TiO2 NPs, uncoated TiO2 NPs, as well as the polymer coating alone. Zebrafish (*Danio rerio*) embryos were exposed to 0.1, 1 or 10 $mg \cdot L^{-1}$ of each NP type and subsequently irradiated with UV light similar to the intensity and spectrum of natural light. We assessed sublethal endpoints including total glutathione levels and superoxide dismutase activity after 7 days and we tested the potential for NPs to interfere with these biochemical assays. While co-exposure of polymer-coated TiO2 NPs and UV light had no effect, uncoated TiO2 NPs significantly increased total glutathione levels at all concentrations tested. These uncoated TiO2 NPs, however, have been shown to absorb at wavelengths monitored in the presented assays, which can lead to an inaccurate assessment of toxicity. To validate these findings, we plan to examine the differential expression of zebrafish glutathione peroxidase 1a and superoxide dismutase 2 genes. Further research is needed to determine if these NPs are phototoxic, and development of new and more accurate endpoints for NP toxicity testing is essential.

Inferring long-term algal dynamics in prairie reservoirs (Saskatchewan, Canada) through environmental DNA in cores of sediments (PL)

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With a variety of man-made factors such as climate change affecting inland waters, the future of water quality in many lakes and reservoirs, including those in the Canadian Prairies, is uncertain. Increased frequencies and/or intensities of algal blooms can mean that costly water purification processes must be engineered to reduce offensive tastes, odors, and algal toxins. Unfortunately, good long-term monitoring data are often lacking for major potable water supplies within the Canadian Prairies. Such information is essential to assess long-term trends in lake and reservoir ecology and environmental quality and to inform resource management decisions. Various paleolimnological techniques have been used to infer long-term trends in the limnology of inland waters. An emerging tool is genetic analysis. DNA released from algae can be preserved in lake sediments. Analysis of this DNA can provide historical information about a water body regarding the presence or absence of particular species or toxin-producing gene sequences. Using Buffalo Pound Lake and Lake Diefenbaker, Saskatchewan, Canada, as case studies, environmental DNA (eDNA) analysis is currently being assessed to reconstruct historical trends in both algal community composition and the abundance of toxin producing genes in potable water supplies. Sediment cores were collected from two sites in Lake Diefenbaker (October, 2011 and August, 2012) and from one site in Buffalo Pound Lake (October, 2013). These cores were sectioned at 1-cm increments and eDNA was extracted from a subset of increments from each core profile using an Omega Biotek E.Z.N.A Soil DNA kit. Organism identification involved the sequencing of the 23S (prokaryotic and eukaryotic algae species) and 16S (cyanobacteria species) rRNA gene regions using an Illumina MiSeq Desktop Sequencer. Overall, cores of sediments contained abundant eDNA ranging between 8.7 ng·μL^{-1} to 85.4 ng·μL^{-1} for Lake Diefenbaker and between 30.1 ng·μL^{-1} to 121.7 ng·μL^{-1} for Buffalo Pound Lake. Average A260/A280 absorbance ratios of extracted eDNA were close to 1.8, indicating good quality DNA samples (little protein contamination). To date, only eDNA from Buffalo Pound Lake sediment has been sequenced. The 16S rRNA sequences revealed 19 different species of algae in Buffalo Pound sediments, some of which are known to produce toxins (e.g., *Dolichospermum* spp., *Microcystis* spp. and *Palnkothrix* spp.). The number of "hits" for these species increased in the upper increments of the sediment core, as determined using Illumina's MiSeq Reporter Software, suggesting either an increased abundance of these species in recent years or gradual degradation of DNA over time (i.e., reduced DNA concentrations down-core). Results of the analysis of 23S rRNA were limited by a lack of information in the NCBI public database, but sequences did reveal the historical presence of five species of cyanobacteria and eukaryotic algae.

Is the development of the innate immune system in *Xenopus laevis* regulated by thyroid hormone? (PL)

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Recent large-scale amphibian population declines have in part been attributed to contaminant-induced immunotoxicity; however, evaluating the impact of contaminants on amphibian immunity is difficult due to the fact that the amphibian immune system is dynamic over development. Endocrine changes associated with metamorphosis, namely the dramatic increase in thyroid hormone (THs) and corticosteroids, are also responsible for cytokine response, loss of larval lymphocytes and other immune system changes as a tadpole transitions into a frog. Therefore, it is possible that contaminants that disrupt endocrine regulation may also compromise immune system development. To better understand cross-talk between thyroid hormone signaling during metamorphosis and larval immune system changes, we focused on pro-inflammatory cytokines - soluble immune mediators that allow communication between components of both the innate and adaptive immune systems. First, developmental expression profiles from early embryogenesis to metamorphic climax were established for three cytokines (interleukins-1β (IL-1 β), tumor necrosis factor α (TNF-α) and interferon gamma (IFNγ)). *Xenopus laevis* tadpoles were sampled at stages that represent early (Nieuwkoop and Faber (NF) stage 9) and late (stage 20) embryogenesis; early (stage 35), mid (stage 44) and late (stage 51) pre-metamorphosis; as well as early pro-metamorphosis (stage 56) and completed metamorphosis (stage 66). Results indicate that the expression of pro-inflammatory cytokines is transient across developmental stages. For all genes, high initial levels of expression suggest maternal transfer of mRNA transcripts followed by a decrease in expression and finally an increase at pro-metamorphosis. We then assessed whether thyroidal modulation would influence on cytokine transcript expression. Stage 51 tadpoles, which are athyroid, were exposed to either thyroxine (0.5 and 2 $\mu g \cdot L^{-1}$) or sodium perchlorate (125 and 500 $\mu g \cdot L^{-1}$) for 7 days. Individuals were assessed for developmental stage, lengthed, weighed and collected for analysis of pro-inflammatory cytokine expression. After 7 days, tadpoles exposed to both concentrations of thyroxine were at more advanced stages of development compared to controls, while tadpoles exposed to sodium perchlorate were significantly decreased in length. These results indicate that pre-metamorphic tadpoles responded to thyroid axis agonist/antagonist exposure, and analysis of cytokine expression will provide insight into whether thyroidal disruption may compromise innate immune system development.

Investigating the potential effects of pollutants on the gastrointestinal microbiome of rainbow trout (*Oncorhynchus mykiss*) (PO)

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The gastrointestinal microbiome influences metabolic processes, plays a role in host nutrition, and contributes to intestinal and immune system development in vertebrates, including fish. Many factors can affect the composition of the intestinal microbiome in fish including diet and host phylogeny. While a number of studies have shown that microorganisms are sensitive to low concentrations of pollutants, to date little to no information is known about the effects of contaminants on the bacterial community composition of the intestines. Any changes in gut microbiome composition have the potential to negatively influence fish health, and due to the essential role of bacteria in the gut, research is needed in this area. Furthermore, as some bacterial taxa are found only in fish, these species could have the potential to be used as bioindicators of pollutant exposure. To further explore the potential impacts of contaminants on the intestinal microbiome of fish, this study investigated the potential effects of two classes of pollutants of environmental concern, namely antimicrobials and polycyclic aromatic hydrocarbons (PAHs), on microbial composition in the gut of rainbow trout. Antimicrobial compounds are extensively used in aquaculture, as well as in humans and animals, to treat bacterial diseases, which can lead to residues entering the aquatic environment and possible subsequent microorganism toxicity. PAHs are ubiquitous in the environment and can elicit a variety of deleterious effects on a wide range of organisms, including bacteria. Gastrointestinal microbiome composition was determined by next generation sequencing of the 16S rRNA gene using an in-house Illumina MiSeq system. This research aims to provide insight into novel aspects of toxicity and chemical metabolism in fish, and further our understanding of the important role played by gut flora in host well-being and adaptation to changing environmental conditions. Furthermore, information generated from this study may be used in identification of bioindicator taxa present in the gut microbiome, and development of biomonitoring strategies around known pollutant sources.

Determination of acute and sub-chronic toxicity of emerging contaminants to early life stages of rainbow trout (*Oncorhynchus mykiss*) (PO)

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In recent years, emerging contaminants have gained notoriety due to their ubiquity in the aquatic environment as well as the lack of data available regarding their toxicity to wildlife and humans. This is particularly relevant in northern climates, where conditions are colder and many fish species are longer-lived, which may make them more susceptible to chronic toxic insults than their counterparts in warmer climates. Furthermore, most data to date has been garnered using standard laboratory species, which may not be particularly relevant considering the potential role of life history, trophic level, physiology, and climate on the species-specific toxicity of chemicals. Emerging chemicals of concern such as hexabromocyclododecane (HBCD), silver (Ag) nanoparticles, short-chain chlorinated paraffin (SCCP), 17α-ethynylestradiol (EE2) and Prozac™ (FLX) primarily enter the aquatic environment as mixtures through municipal wastewater effluents (MWWEs). MWWE, which is typically a mixture of industrial, commercial, and household wastes, may be released into receiving waters with little to no treatment, which is not uncommon, especially in rural Canadian municipalities. These chemicals may then sequester to sediments or expose biota through the water column. In this study, fertilized *Oncorhynchus mykiss* gametes were obtained and exposed to six concentrations of EE2, FLX, MWWE, and Ag nanoparticles through water, whereas SCCPs and HBCD were exposed through sediments. For each chemical, the lowest concentrations were selected based on environmental relevance and increased incrementally thereafter, with the highest doses encroaching on acute toxicity thresholds. Fish were exposed under continuous flow-through conditions and subsamples were collected at critical development stages to assess acute and chronic toxicity of all test chemicals. Endpoints included hatching success, time to hatch, survival, time to swim-up, time and success of sexual differentiation, and morphological and histological defects, as well as endpoint-specific rates of development. We hypothesize that the chronic exposures to early life stages of *O. mykiss* will have different toxic effects than the acute exposures, from which most chemical characterizations are derived, and that the selected emerging contaminants will exert life stage-specific toxicity. Future research will aim to characterize the effects of these emerging chemicals on other native species including white sturgeon (*Acipenser transmontanus*), lake trout (*Salvelinus namaycush*), northern pike (*Esox lucius*), and fathead minnow (*Pimephales promelas*) in order to aid in the development of more appropriate environmental risk assessment strategies as well as to potentially establish a guide for interspecies comparisons.

Chemodynamic behaviour of thallium in the Slave River, Northwest Territories, Canada (PO)

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Environmental dynamics and effects of thallium (Tl) are probably the least understood of any of the metals that occur as contaminants in aquatic systems. The primary sources of Tl to the environment include extraction and combustion of fossil fuels and potassium based fertilizers. In this study concentrations of metals in the muscle tissue of fish from the Slave, Peace, and Athabasca rivers were quantified by digestion with nitric acid and hydrogen peroxide followed by use of ICP-MS. Concentrations of Tl in dorsal muscle of certain fishes in the Slave River were greater than those in the same tissues and species in the Peace and Athabasca Rivers. These greater concentrations of Tl were most noticeable in higher trophic species such as northern pike (*Esox lucius*) and walleye (*Sander vitreus*), but to a lesser extent in lower trophic level fishes, such as whitefish (*Coregonus clupeaformis*). Mean concentrations of Tl in muscle of northern pike, stratified by season, were 16, 7.8, and 11 $\mu g \cdot kg^{-1}$ (dry mass (dm)) furthest upstream, compared to 55, 39, and 60 $\mu g \cdot kg^{-1}$ dm furthest downstream. Mean concentrations of Tl in walleye, stratified by season were 19, 27, and 24 $\mu g \cdot kg^{-1}$ dm furthest upstream compared to 53, 79, and 89 $\mu g \cdot kg^{-1}$ dm furthest downstream. Mean concentrations of Tl in whitefish, stratified by season were 7.3, 6.9, and 6.3 $\mu g \cdot kg^{-1}$ dm furthest upstream compared to 17, 18, and 16 $\mu g \cdot kg^{-1}$ dm furthest downstream. The presumed source of Tl in the Slave system is operations in Alberta that are extracting and upgrading bitumen in oil sands. However, it is unclear why concentrations of Tl were greater in fishes from the lower Slave River than those in fishes of the other two rivers. It is possible that differences in oxidation state or other speciation phenomena that could affect bioavailability occur in the upper Slave River which could result in differential accumulation efficiencies between the upper and lower stretches of the Slave River. Investigations into these possible speciation changes are currently ongoing. The greater concentrations observed in species of higher trophic level suggests there is potential for biomagnification of an organic form of Tl, possibly dimethyl thallium. While concentrations of Tl in tissues of fishes would not be toxic to the fishes or consumers, including humans, this phenomenon offers an opportunity to further investigate the environmental chemodynamics of this poorly understood element.

Direct and indirect toxicity of single-walled carbon nanotubes to freshwater invertebrates (PO)

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Production and development of carbon nanomaterials has increased for the past few years. In Canada, the forestry industry experienced an economic crisis which has led to the elaboration of new forestry products: nanomaterials, including single-walled carbon nanotubes (SWCNT). Because carbon nanomaterials have a large specific surface area giving them high adsorption capacity, they are closely studied for drug targeting and water treatment. Indeed, this property is very interesting for the removal of contaminants like metals in wastewater. Adsorption of metals is greater for purified or functionalized carbon nanotubes. Production increase of nanomaterials and applications for water treatment raises the question of their direct but also indirect toxic effects on aquatic organisms which may be exposed. The adsorption properties of SWCNT may alter the bioavailability of contaminants present in the medium and change the global toxicity of the mixture. This research focuses on direct and indirect toxicity of single-walled carbon nanotubes (SWCNT) to freshwater organisms. First, we measured the toxicity of SWCNT to the amphipod *Hyalella azteca* and the influence of sediment presence. We observed a decreased toxicity in presence of sediment, which suggests that SWCNT are adsorbed on the organic matter from the artificial sediment, leading to a decrease of SWCNT bioavailability. Secondly, we evaluated the toxicity of SWCNT caused indirectly by the adsorption of a toxic metal (Cd), using the crustacean *Daphnia magna* and 48-hour lethality tests. We found that SWCNT adsorbed Cd and that the presence of SWCNT increased the toxicity of Cd to *D. magna*. Our results suggest that the increase in toxicity is due to the adsorption of Cd on carbon nanotubes which induces a higher uptake of Cd in the digestive tract of *D. magna*. Then, once entered into the digestive tract, Cd can be desorbed thanks to the enzymatic conditions contributing to desorption processes. Finally, we measured the immunotoxic and genotoxic effect of SWCNT to the freshwater mussel *Elliptio complanata* alone or in the presence of Cd. Higher DNA damages were measured in hemocytes from mussels exposed to SWCNT with Cd than to each contaminant alone. Those results show the complexity of SWCNT toxicity and how the understanding of their interactions with other contaminants is crucial to determine the environmental impacts of their release in the environment.

Presence and toxicity of microplastics in the Canadian aquatic environment: A review (PO)

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Microplastics are increasingly detected in various aquatic environments, and there are growing concerns that they can have toxic effects when ingested by biota in Canadian freshwater and marine environments. An extensive literature review was performed by Stantec Consulting Ltd. on behalf of Fisheries and Oceans Canada, in an effort to summarize the current state of knowledge of microplastics in marine and freshwater environments. The first specific objective was to summarize: 1) the sources and composition of microplastic materials in the aquatic environment, 2) their environmental fate and behaviour, including previously reported environmental levels of various size classes in surface waters, water column, and sediments, and 3) pathways of ingestion by biota (direct ingestion and trophic transfer) and persistence in tissues. The second objective was to summarize the toxic potential of microplastics in aquatic biota, including invertebrates, fish, and marine mammals. Included was an analysis of the types of contaminants that can associate with microplastics (i.e., intrinsic components of the plastic material itself), as well as other environmental contaminants (such as polychlorinated biphenyls (PCBs) or other persistent organic pollutants (POPs)) that adsorb or bind to the plastic. The toxic effects of microplastic-associated contaminants were also considered. The third specific objective was to identify knowledge gaps and specific needs for future research to better evaluate the aquatic toxicity of microplastics. Identified gaps include further identification of sources, behaviour and toxicity of microplastics and their associated contaminants, particularly in freshwater environments and the Arctic.

Predicting energy content and fish condition of juvenile rainbow trout (*Oncorhynchus mykiss*) using bioelectrical impedance analysis (PO)

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Determining the body composition and condition of fish is an important aspect of aquaculture and fish ecotoxicology research. Traditional destructive methods are time consuming, costly, and usually result in the loss of the animal. Bioelectrical impedance analysis (BIA) is a non-lethal method which, once validated, can be used to quickly and accurately determine the body composition and condition of fish. In this study, rainbow trout (*Oncorhynchus mykiss*) were subjected to one of three different 90-day feeding regimens: maintenance (0.4% body weight), optimal (1.6% body weight) or satiation (3.4% body weight). Fish were sampled from each feeding group at 30 days, 60 days and 90 days into the experiment. Models predicting the total body water (R^2=0.99) dry mass (R^2=0.98) and energy content (R^2=0.96) of rainbow trout were developed using BIA and validated with traditional bomb calorimetry. Condition of the fish was determined using

bioelectrical impedance vector analysis (BIVA) which uses a plot of the reactance and resistance of the fish normalized against the detector length. BIVA showed that fish fed on a satiation diet (3.4% body weight per day) were distinct from fish fed on a maintenance diet (0.4% body weight per day). These findings suggest that BIA can be used to determine the body composition and condition of rainbow trout with high accuracy in a non-lethal manner. BIA thus allows for repeated measurements of a fish which will facilitate future energetics and growth studies on rainbow trout.

Predicting the bioaccumulation of organic sunscreen agents in rainbow trout (*Oncorhynchus mykiss*) using measured *in vitro* biotransformation rates (PO)

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Organic ultraviolet filters (UVFs) are ingredients common in sunscreens and other personal care products and may enter the aquatic environment directly from the skin during swimming and other recreational activities or indirectly via wastewater effluents. Select UVFs are hydrophobic (log K_{OW}>4.0) and have been widely detected in surface waters and aquatic biota, thus there are concerns about their potential to bioaccumulate in organisms. Bioaccumulation is typically assessed using the octanol-water partition coefficient (K_{OW}) and/or bioconcentration factor (BCF) in fish. Although BCFs of select UVFs fall below Canadian regulatory criteria (BCF<5,000), their bioaccumulation in higher trophic level organisms and in aquatic food webs in the field has been observed. Further investigations on UVF bioaccumulation potential are warranted to investigate these discrepancies. In addition to these discrepancies, there are few empirical BCFs available for UVFs, suggesting additional data is needed. Because biotransformation can reduce chemical bioaccumulation in fish and in food webs, bioaccumulation models using biotransformation rates from fish to predict chemical BCF values have been developed. Biotransformation rates may be measured by *in vitro* followed by *in vitro* to *in vivo* extrapolation methods to obtain whole organism (*in vivo*) biotransformation rates and chemical BCF values. To determine if UVFs are biotransformed in fish, the present study investigated the *in vitro* biotransformation of select organic UVFs, including 2-ethylhexyl-4-trimethoxycinnamate, avobenzone, and 4-methylbenzylidene camphor. The effect of chemical concentration on the *in vitro* biotransformation rate was also investigated to obtain enzyme kinetic parameters. *In vitro* biotransformation rates were extrapolated to organism level *in vivo* rate constants and modeled BCF values. BCFs predicted by the model were then compared to *in vivo* BCFs and BCFs predicted without biotransformation. UVF BCF values that consider *in vitro* biotransformation rates were closer to empirical BCFs than when biotransformation was not considered. This study demonstrates the value of integrating measured *in vitro* biotransformation rates for prediction of chemical bioaccumulation in fish.

Pollution-induced effects in feral fish from the river Holtemme assessed by biomarker analysis (PO)

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In a water quality report published in 2005 most German rivers were predicted to fail to achieve good ecological status without profound improvements. One river that has been classified by this report as having poor water quality was the river Holtemme in Saxony-Anhalt. Therefore, the aim of this study was to assess the contamination levels and potential resulting ecotoxicological effects on aquatic organisms in the river Holtemme. To that end, selected biomarkers for genotoxicity, dioxin-like activity and endocrine disrupting effects were analyzed in samples of wild brown trout (*Salmo trutta*) collected from the river. Four sampling sites were chosen to determine effects of specific point sources of exposure, such as agricultural use or discharges of waste water treatment plant (WWTP) effluents. Significantly increased hepatic 7-ethoxyresorufin-O-deethylase (EROD) activities could be observed at two study sites downstream of WWTP effluent discharges. Moreover, a potential to cause genotoxic effects could be demonstrated as determined by a significant increase in the micronucleus frequency in fish collected from the same locations. Analysis of plasma 17β-estradiol and testosterone concentrations showed no significant differences among sampling sites, although a slight trend towards elevated hormone concentrations at the last study site in an agriculturally used area was observed. Chemical analysis of water samples revealed elevated conductivities as well as concentrations of heavy metals and nitrogen compounds in areas that are located downstream of WWTP effluent discharges. However, contaminant concentrations and biomarker effects were not correlated. In this study, significant differences between biomarker responses could be demonstrated for both urban and agricultural areas. Hence, adverse environmental impacts on aquatic but also other organisms caused by anthropogenic land use near the river Holtemme cannot be excluded. Ongoing studies currently characterize the site-specific molecular toxicity pathways in the same fish by use of sequence-by-synthesis technology, with the ultimate goal to identify candidate contaminants groups responsible for the biological effects observed as well as to predict potential adverse outcomes.

In vitro characterization of the toxicity of selected emerging contaminants to lake trout (*Salvelinus namaycush*) (PO)

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There is an ever-increasing number of chemicals including pharmaceuticals and industrial pollutants released into the aquatic environment, leading to the exposure of fish and other aquatic life. To date, there is limited knowledge about the potential effects and the sensitivity of native fish species in North America to these emerging chemicals. However, given the huge number of compounds that will have to be tested, there is a need to address the ethical concerns regarding the large number of animals required for current standard *in vivo* toxicity testing, particularly when working with protected and endangered species. Therefore, the aim of this present study was to investigate the potential toxicity of selected emerging chemicals of concern, namely 17α-ethinylestradiol (EE2), Prozac™, and silver nanoparticals (AgNPs), to lake trout (*Salvelinus namaycush*) using *in vitro* gonadal and liver explant assays. Gonadal explants were used to investigate disruption of sex-steroid hormone synthesis upon exposure to these compounds. Liver explants were used to investigate the general toxicity of these compounds as well as their effects on selected pathways including metabolism, oxidative stress and endocrine disruption. Lake trout were sampled in northern Saskatchewan, gonads and liver were excised, sliced into $1 mm^3$ pieces and placed into a 24-well cell culture plate containing supplemented L-15 media. Tissue explants were exposed to increasing concentrations of forskolin, prochloraz, EE2, Prozac™, or AgNPs, along with a solvent control (DMSO) for 24 hours. Hormone concentrations in media were quantified using ELISA. Abundance of various transcripts from exposed liver explants were quantified using q-PCR. This study is part of a larger ongoing research effort that investigates the toxicity of the above compounds to four other species native to Canadian freshwater systems (rainbow trout (*Oncorhynchus mykiss*), lake whitefish (*Coregonus clupeaformis*), northern pike (*Esox lucius*), white sturgeon (*Acipenser transmontanus*)) using *in vitro* (featured here) as well as early life stage *in vivo* approaches, which will be used to characterize the sensitivity of these species to these chemicals.

Evaluation of genotoxic substances in zebrafish embryos using the fish embryo toxicity and micronucleus assays (PO)

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There is increasing concern over the number of pollutants that enter the aquatic environment and disrupt reproduction, growth and survival at the population and ecosystem level. One class of compounds of particular concern is genotoxic substances. These substances enter the aquatic environment through varied sources, including point sources such as municipal and industrial effluents and diffuse sources such as atmospheric deposition of combustion products; all leading to the exposure of aquatic life. The fish embryo toxicity (FET) test is a common bioassay used to identify the potential for a chemical or environmental sample to cause teratogenic effects. In addition, the FET method is amenable to the assessment of additional mechanism-specific endpoints. Another common test used to investigate genotoxicity is the micronucleus (MN) test, in which micronuclei created during cell division are assessed in the cytoplasm. Micronuclei formation can be induced by chromosome fragments or whole chromosomes that are unable to migrate to the poles because of spindle damage. The aim of this study was to apply the MN assay with 5 days post-fertilization zebrafish larvae (*Danio rerio*) after an exposure to two well-known genotoxic compounds, 4-nitroquinoline-1-oxide (NQO) and cyclophosphamide (CPP). NQO is a direct-acting genotoxic agent and can induce DNA damage through the generation of reactive oxygen species. CPP acts as an indirect genotoxicant, which needs a metabolic activation by oxidase. Both are used as positive controls in the MN assay, since they are known to induce modifications in the DNA, leading to micronuclei in the cytoplasm. Zebrafish eggs were exposed for 120 hours to different concentrations of NQO or CPP, along with water and solvent controls. Following the exposure, cells of the whole larvae were dissociated by mechanical and enzymatic digestion. The cell suspension was then fixed on microscope slides and the micronuclei in each sample were quantified. Molecular endpoints were also investigated through the evaluation of gene expression by qPCR. Transcript abundance of cytochrome P450 1A, p53, p21, and mdm2 were quantified on a subset of samples collected at 48 and 72 hours during the exposure. A more comprehensive analysis of potential genotoxicity can be conducted though the combined assessment by both of these tests, providing information at the molecular, cellular and organism level.

Mechanisms affecting arsenic flux rates from prairie reservoir sediments, Buffalo Pound Lake, Saskatchewan, Canada (PO)

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Buffalo Pound Lake, Saskatchewan, is a eutrophic reservoir that provides potable water to the nearby major cities of Regina and Moose Jaw. Lake sediments are hypothesized to be releasing nutrients into the overlying water and driving annual blooms, while simultaneously releasing associated arsenic (As). Although the magnitude of seasonal release of As from Buffalo Pound Lake sediment does not represent a human or animal health risk, it provides an opportunity to assess potential mechanisms of As release in prairie reservoir sediments. Using sediment core incubation experiments, this study investigated the effects of oxygen status and temperature on arsenic and phosphorous mobilization from Buffalo Pound Lake sediments. Sediment cores from four sites (cores collected June 2014) were incubated under either oxic or anoxic conditions for 35 days at 20°C, simulating summer conditions. Overlying water was analyzed for dissolved As, iron, manganese, and phosphorus fractions. A second core incubation experiment (cores collected October, 2014) was conducted using a similar design with cores from only two of the sites incubated for 105 days at 4°C, simulating winter conditions. Flux rates for each treatment and site were calculated and statistically compared using two-way ANOVA. During the simulated summer incubation, As release did not significantly differ between oxic (0.61 ± 0.27 mg/m^2/day, $n=12$) and anoxic (0.73 ± 0.30 mg/m^2/day, $n=12$) treatments ($p>0.05$). However, site location significantly affected As flux rate ($p<0.001$). Arsenic remobilization under anoxic conditions was likely due to dissolution of sediment-associated iron and manganese oxyhydroxides, and release of associated As into overlying water. Arsenic remobilization under oxic conditions was likely a result of the formation of anoxic conditions below a thin oxic sediment-water interface, and the inability of this layer to scavenge As diffusing upward. Site differences in As release were likely due to differences in sediment geochemistry and As inventory, both of which are currently being assessed. During the simulated winter incubation, the concentrations of dissolved arsenic did not rise above background concentrations, regardless of oxygen treatment, and flux rates were not calculable. It is hypothesized that both microbial and macroinvertebrate activity are significantly inhibited at 4°C, both of which influence As flux and remobilization. Overall, the release rate of As from Buffalo Pound Lake sediments, for a given site, appears to be more dependent on temperature rather than oxygen status. Additional core incubations will be conducted to investigate the effects of alkaline pH waters and sediment microbial and macroinvertebrate activity on As remobilization and flux rates. *In situ* work will be completed to assess real-time As dynamics and to validate laboratory data.

Mitigating chronic arsenic poisoning in a Bangladeshi community using high-selenium lentils: Planning and design of a clinical trial (PO)

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An estimated 100 million people worldwide are chronically exposed to unacceptably high concentrations of arsenic in their drinking water and food supply. Since tube well water contamination by arsenic was discovered in Bangladesh in the 1990s, levels in drinking water have been reduced by 40%, yet approximately 45 million Bangladeshis remain at risk from high arsenic concentrations in their well water (World Health Organization Fact sheet N°372, December 2012). Soils deficient in selenium, as is the case in Bangladesh and many other areas of the world, produce crops that are low in selenium, which becomes a "partner in crime" exacerbating the toxic problems associated with arsenic exposure. Selenium is an antagonist to arsenic toxicity, and supplementation of selenium may protect from adverse health effects caused by arsenic poisoning. High-selenium lentils may be a cost-effective and practical solution, as lentils are a staple crop in Bangladesh. We have planned a 6-month clinical trial in a community of Bangladesh, where arsenic levels in household water exceed the national standard (>50 ppb). This double-blind, randomized clinical trial is commencing in October 2015. Recruited families will be eating high-selenium lentils from Saskatchewan, Canada, which will deliver the recommended daily allowance of 55 µg/day/person. The control group will receive low-selenium lentils. 200 individuals will be recruited for each treatment group. Both types of lentils for this trial were grown during the summer of 2014. Village health workers will distribute the lentils weekly and will assess compliance of lentil consumption with a questionnaire. Morbidity data will be collected every 2 weeks via questionnaire. The families will visit the field office for medical examination and collection of blood, urine, stool and hair specimens 3 times during the dietary trial, at Day 1, at 3 months and at the end of the trial. Specific aims of the trial: 1) To determine if high-selenium lentils can increase the urinary and fecal As excretion, 2) To determine the efficacy of high-selenium lentils in reducing As levels in the hair of individuals, 3) To determine the effect of high-selenium lentils on anti-oxidant status in blood (ratio of reduced glutathione (GSH) to oxidized glutathione (GSSG) and their potential to reduce oxidative stress (8-hydroxy-2′-deoxyguanosine (8-OHdG) in blood), 4) To determine if high-selenium lentils can modulate the lipid profile in serum, 5) To determine if high-selenium lentils can reduce arsenic-related lung inflammation (determined through exhaled fractional NO).

Development of a novel biological marker of endocrine disruption in rainbow trout (PO)

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The incidence of endocrine disrupting chemicals (EDCs) in the environment is a growing concern and sublethal toxicity is not easily detected. Although EDCs can affect any aspect of the endocrine system, they are generally identified through reproductive endpoints in fish. However, identification of EDCs that affect other aspects of the endocrine system is more difficult. Thyroid hormone (TH) plays a key role in many physiological processes, including growth, neural development and reproduction, yet disruption of the signaling pathway for this vital hormone has not received much attention, possibly due to the difficulty in assessing TH disruption in fish. Salmon and trout possess ultraviolet-sensitive (UVS) cones in the retina that enable the detection of UV light and are likely used for navigation and feeding. During development, salmon and trout lose SWS1 opsin expression (involved in UV detection) and the corresponding ability to detect wavelengths in the UV range. This developmental process appears to be regulated by TH, as T4 treatment initiates premature downregulation of SWS1 opsin expression and propylthiouracil (PTU, preventing T4 production) mitigates natural SWS1 opsin gene downregulation. Thus, this link between TH and SWS1 opsin expression could prove to be an effective indicator of thyroid hormone action and its disruption by environmental chemicals. The objectives of this study were 1) to determine the sensitivity of SWS1 opsin downregulation to T4 treatment, and 2) to evaluate the effectiveness of changes in SWS1 opsin gene expression in the retina to identify TH-disrupting chemicals bisphenol A (BPA). To investigate the sensitivity of SWS1 opsin downregulation by T4, 10 juvenile rainbow trout (*Oncorhynchus mykiss*) per treatment were exposed to four concentrations of 0-100 $ng \cdot mL^{-1}$ of T4 delivered via an implant for 4 weeks. Downregulation of thyrotropin hormone (TSH) gene expression in the pituitary indicated the effectiveness of the T4 doses, and preliminary data suggests that SWS1 opsin gene expression was downregulated at 50 $ng \cdot mL^{-1}$. Thus, this process appears to respond to physiological levels of TH in rainbow trout. To test the use of SWS1 opsin expression in evaluating potential TH disrupting compounds, juvenile rainbow trout were exposed to daily renewal of aqueous control, T4, PTU and two BPA treatments (20 & 200 $\mu g \cdot L^{-1}$). After 4-week treatment, the T4-treated group exhibited downregulation of TSH and SWS1 gene expression. The PTU treated group resembled control fish, but had elevated TSH levels. BPA-treated exhibited downregulation of TSH similar to T4 treatment, but did not appear to significantly affect SWS1 expression, suggesting TH disrupting activity. These results suggest that SWS1 opsin downregulation responds to physiological concentrations of T4 in rainbow trout, and that in concert with other elements involved in TH signaling, T4-dependent SWS1 gene expression in the trout retina may be a useful tool to identify TH-disrupting chemicals.

In vivo effects of intravenously injected polymer-coated TiO_2 nanoparticles on goldfish (*Carassius auratus*) immunity (PO)

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Evidence of nanoparticle (NP) effects on immune cell function has been demonstrated using *in vitro* models in previous studies that report both the over-activation of pro-inflammatory responses, and suppression of others. Our group has also demonstrated modified immune effects for both cell lines and isolated primary goldfish neutrophils when exposed *in vitro* to polymer-coated metal-oxide NPs. However, the translation of these effects to *in vivo* models has not yet been explored. Despite a lack of information on *in vivo* immune effects, it has been shown that when NPs enter circulation in fish, the vast majority is deposited into kidney tissues, which in fish are the major hematopoietic organ and where many innate immune cells reside. Thus, there is an increased opportunity for neutrophils and macrophages in the kidneys to interact and be affected by tissue-accumulated NPs. In this study we have isolated kidney neutrophils and macrophages at 0, 1, 7 and 14 days from mature goldfish (*Carassius auratus*) injected on Day 0 with either polymer-coated TiO_2 (1 $\mu g \cdot g^{-1}$) or to Cortland's saline (control). At each time point, kidney, spleen, blood, kidney neutrophils and macrophages were extracted for measurement of tissue metal distribution, expression of various immune and apoptotic-related genes, and neutrophil degranulation and respiratory burst. Peripheral blood and tissue prints were also collected for analysis of white blood cell (WBC) proportions. Preliminary results show increased neutrophil degranulation and respiratory burst relative to control. Gene expression, tissue metal distribution and WBC proportional results will be presented.

Selenium Ecotoxicology

Evaluation of selenium sensitivity of spotted sandpipers breeding in the Elk River watershed of southeastern British Columbia (PL)

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In 2013 and 2014, a study of spotted sandpipers (*Actitis macularius*) nesting in more than 30 areas in the Elk River watershed was completed to determine the potential for selenium-related effects on hatching success. When each nest was found, one egg was randomly sampled for selenium analysis and to determine hatch date. Eggs remaining in the nest after hatch were also collected for examination of egg contents and selenium analysis. Over the two year study, slightly more than half of the 211 nests found were incubated to hatch, approximately one-third were depredated, and 12% were abandoned. A few nests were inundated by rising water levels, while others were trampled by wildlife or destroyed by machinery. Of the 350 eggs that were fully incubated, 17 did not hatch, and of these, only 4 perished for unknown reasons (others appeared to be infertile or abandoned late in development). No clutches incubated to term experienced complete nest failure. General population characteristics (e.g., percent nest success, clutch size and egg hatchability) were nearly identical to those observed by Oring *et al.* (1983) in a multi-year population study of spotted sandpipers in an undisturbed area of Minnesota. Selenium concentrations in eggs collected over the two year study ranged from 1.9 to 32 $\mu g \cdot g^{-1}$ dry weight (dw). The highest selenium concentrations were associated with nests located near lentic areas such as settling ponds, with several having concentrations above the widely-reported 12 $\mu g \cdot g^{-1}$ dw threshold for sensitive bird species. In contrast, selenium concentrations in all spotted sandpiper eggs collected from lotic areas were below 12 $\mu g \cdot g^{-1}$ dw. In areas where sandpipers had access to prey items that varied widely in selenium concentrations, both inter- and intra-clutch variability in egg selenium concentrations tended to be higher. No relationship was found between hatch success and selenium concentrations in eggs ($p=0.386$), nor were nests with egg selenium concentrations above 12 $\mu g \cdot g^{-1}$ dw more likely to contain unhatched eggs ($p=0.556$). Overall, the results of this study demonstrate a very low potential for selenium-related effects on spotted sandpipers nesting in the Elk River watershed.

Concentration dependence and interspecific differences in selenium partitioning between fish tissues (PL)

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Selenium can impair the development and survival of fish embryos, making egg selenium concentrations the most relevant measure for evaluating effects on fish. However, muscle and whole body sampling are more practical for monitoring. Therefore, methods are needed to translate selenium concentrations between tissues. Previous approaches have assumed a constant ratio of concentrations between tissues. We used regression analysis to test this assumption by evaluating the concentration dependence of conversion factors for 30 freshwater fish species from across North America. Conversion factor ratios were typically between 1:1 and 3:1 for most species, although some species exhibited consistently higher ratios (e.g., mountain whitefish (*Prosopium williamsoni*)). In some cases, concentration dependence was apparent, indicating that a constant ratio may not provide an accurate translation.

What do we know about the effects of dietary selenomethionine on white sturgeon (*Acipenser transmontanus*)? (PL)

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The white sturgeon (*Acipenser transmontanus*) is a unique species endemic to Western North America. These fish have a long history of importance to some First Nations peoples as well as an economic importance to modern-day fishing industries. Unfortunately the past decades have seen a dramatic decline in numbers and the species is now listed as endangered. One cause for concern is the increasing levels of industrial pollutants, such as selenium, within white sturgeon habitats. Selenium, while a natural and essential element, can accumulate in common prey items of white sturgeon to concentrations that are toxic to other fish species. Selenomethionine (SeMeth) is the most bioavailable and toxic form of selenium in natural habitats. With the advent of stable white sturgeon cultures, controlled dietary SeMeth exposures can be conducted in the laboratory. So what do we know about the effects of SeMeth on this endangered fish species? This review will compare findings from dietary SeMeth studies conducted at the University of California Davis campus and the University of Saskatchewan, which used two geographically distinct populations. Differences in rates of mortality and organ pathologies (histology), similarities in accumulation rates and growth effects and other findings will be discussed. Lowest observable adverse effects concentrations between white sturgeon and other species will also be compared.

Assessing the potential effects of chronically elevated dietary selenium exposure on the fitness of the model amphibian *Xenopus laevis* (PL)

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Selenium (Se) is a contaminant of potential concern in aquatic systems due to its efficient incorporation into food webs, potential for bioaccumulation at higher trophic levels, and role as a developmental toxicant in oviparous vertebrates. While embryonic/larval deformities are considered the most sensitive toxicological endpoint, elevated levels of dietary Se have also been associated with alterations to bioenergetic and hormonal status of adult female fishes, which consequently could lead to diminished fitness and impaired reproduction. Adverse physiological and behavioural effects in fish have been the primary focus of Se research; however, studies focusing on Se toxicity in amphibians in any regard are severely lacking. The objective of this study was to investigate the possible ecological risk associated with chronically elevated dietary selenium consumption in adult female amphibians utilizing the model species *Xenopus laevis*. Adult *X. laevis* females (n=9-10) were fed a diet augmented with L-selenomethionine at measured concentrations of 0.7 (control), 10.9, 30.4, and 94.2 μg $\mathrm{Se \cdot g^{-1}}$ dry mass (dm) for 68 days, after which they were bred with untreated males. The resultant embryos were incubated for 5 days post-fertilization (dpf) to determine fertilization success, hatchability, mortality and frequency of malformations. Ovary, egg, liver, muscle and blood samples were collected from *X. laevis* females upon completion of the exposure period and subsequent breeding to ascertain Se tissue distribution, triglyceride and glycogen levels, and cortisol concentrations. Reduced/oxidized glutathione concentrations, and lipid peroxidation levels on 5 dpf tadpoles were analyzed to determine the potential effects on antioxidant defense mechanisms and their relation to Se-induced malformations. Survival, hepatosomatic index, and gonadosomatic index of adult female *X. laevis* after exposure to dietary Se exposure showed no significant differences among treatment groups, though a trend towards female weight loss was observed with increasing dietary Se concentrations over the 68 days ($p=0.063$). The concentration of Se present in eggs increased in proportion with rising maternal dietary intake with measured values of 1.6, 10.8, 28.1 and 81.7 μg $\mathrm{Se \cdot g^{-1}}$ dm, respectively. No biologically significant effects were observed regarding fertilization success, hatchability or mortality; however, a significant increase in the frequency and severity of morphological abnormalities was observed in 5 dpf tadpoles from the highest dose group when compared to the control ($p<0.001$). Overall, this study aims to aid in further understanding the sensitivity of amphibians to Se, with the goal of assisting in the development of environmentally relevant toxicity thresholds.

Community composition matters: Selenium accumulation in periphyton and trophic transfer to the freshwater snail, *Stagnicola elodes* (PL)

Lorne Doig[1], Vanessa Pittet[2], Blue Markwart[1], Monique Haakensen[2] and Karsten Liber[1]

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The main source of uncertainty in modelling Se distribution in aquatic food webs lies in the enrichment factor (EF), the first step of the food web that involves bioaccumulation of Se from the abiotic environment. EFs can vary dramatically among algal taxa. The majority of bioaccumulation data have been generated from single-species lab tests. It is unclear how periphyton community composition influences the uptake of abiotic Se and subsequent transfer to other trophic levels. The goal of this study was to assess whether periphyton community composition influences the uptake of waterborne Se (as selenite) and subsequent transfer to macroinvertebrate primary consumers. This was accomplished through a simplified food chain experiment (water–periphyton–snail) involving different communities of periphyton and the herbivorous freshwater snail *Stagnicola elodes*, in combination with targeted metagenomics analysis of periphytic communities raised under different conditions. Periphyton biofilms were grown in aquaria inoculated with filtered (0.35 µm mesh) pond water (Saskatoon, Saskatchewan) and approximately 20 µg Se·L^{-1}(measured concentrations were 21.0 ± 1. µg Se·L^{-1}) added as selenite. Five different light and nutrient regimes (three replicates per treatment) were then used to grow different periphytic communities in the aquaria. After six weeks of maturation of the periphytic communities, 80 cultured snails (0.5 mm to 5 mm in length) were added to each aquarium. Samples were collected for Se analysis as follows: water (days 0, 3, 7, 14 and 21), periphyton (days 0 and 21), and snails (days 3, 7, 14 and 21, whole-body Se). Periphyton samples were collected in triplicate from each replicate tank on Day 0 and Day 21 for targeted microbiome sequencing analysis (Contango Strategies Ltd., using Illumina sequencing platform). The nutrient and light treatments resulted in substantially different periphytic biofilms, with each being consistent across replicates and through the study. Both the LL (61.8 ± 12.1 µg Se·g^{-1} dry weight (dw)) and HL (30.5 ± 4.7 µg Se·g^{-1} dw) biofilms, high in Cyanobacteria content, had statistically higher concentrations of Se relative to the other treatments (which ranged from 13.4 ± 4.1 µg Se·g^{-1} dw to 20.6 ± 2.2 µg Se·g^{-1} dw). Interestingly, the treatment with the highest periphyton Se concentration also had the lowest snail whole-body Se concentrations at the end of the study. Snails in that treatment (0.53 mg/snail) were very small compared to the other treatments (ranging from 3.73 to 6.50 mg/snail) and suggest the possibility of selective grazing by the snails. Periphyton community composition can therefore affect not only the EF, but also the TTF through food selectivity by primary consumers.

Legacy effects of coal combustion residuals on freshwater ecosystems in North Carolina (PL)

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The coal combustion processes involved in electrical power production generate several waste products, including fly and bottom ash, flue-gas desulfurization solids, and boiler slag. Collectively termed coal combustion residuals (CCRs), these waste products have a substantial footprint: in 2013, approximately 114.7 million tons of CCRs resulted from coal consumption activities in the United States (U.S.) alone. Despite being elevated in several toxicologically relevant contaminants (e.g., arsenic, cadmium, and selenium (Se)), CCRs are not classified as hazardous waste by the U.S. Environmental Protection Agency and are discharged into public rivers and lakes via permitted effluent streams. Further, CCR contaminant levels in diluted effluent streams and receiving waters do not adequately represent the potential for toxicological consequences in aquatic ecosystems that result from contaminant enrichment and retention in sediments and sediment pore waters where they can bioaccumulate in system biota. The overarching aim of this ongoing research project is to understand the legacy-specific consequences of CCR contaminants in lentic freshwater systems that receive, or have historically received, CCR effluents. This work involves six lakes in North Carolina, including three CCR-impacted lakes and three reference lakes matched on the basis of trophic status. Three supporting objectives serve to address the primary aim: (1) to determine CCR concentrations in selected fish tissues collected from CCR-impacted and reference sites and determine whether there are differences attributable to site trophic status; (2) to examine the extent to which CCR-specific chemical signatures are apparent in aquatic ecosystems; and (3) to measure CCR uptake, trophic transfer, and toxicity in freshwater biota. Throughout the project, specific emphasis is placed on Se contamination due to the element's unique ability to persist in aquatic systems, substantially enrich to bioaccumulative forms in at the base of the food chain (i.e., an enrichment factor in algae ranging from 102-106), trophically transfer to prey and predator species, and cause toxicity to oviparous vertebrates. Progress to date will be shared at this meeting. Results will include contaminant profiles of surface and sediment pore waters, field-collected biofilms, and fish tissues (liver, muscle, gonad) measured by inductively coupled plasma mass spectrometry (ICP-MS). Boron and strontium isotopic signatures (δ11B and 87Sr/86Sr) previously identified in CCR-impacted environmental media will also be characterized in surface and sediment pore waters, biofilm, and fish bone samples by thermal ionization mass spectrometry (TIMS). Lastly, results of developmental toxicity assays with field-collected pore water exposures to embryonic zebrafish (*Danio rerio*) will be discussed. This work was supported by a grant from the Water Resources Research Institute of UNC and NIEHS Award Number T32ES021432.

Effects of chronic dietary exposure to selenomethionine on the transcriptome of juvenile white sturgeon (PO)

Sarah Patterson[1], Jenna Zee[1], Song Tang[1], Steve Wiseman[1] and Markus Hecker[1]
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White sturgeon (*Acipenser transmontanus*, WS) are an ancient species of fish endemic to western North America. Unfortunately, their populations have been declining since the 1800s. Due to their longevity and benthic lifestyle, WS might be particularly susceptible to bioaccumulative toxicants. Selenium (Se) is an essential micronutrient, but can be highly toxic to vertebrates, including fish, at slightly elevated concentrations. The organic form of Se, Selenomethionine (SeM), is the major dietary form to which aquatic organisms are exposed and has become a particular environmental concern as it persists, bioaccumulates, and biomagnifies. SeM can affect various physiological processes in fish but there is limited understanding in regards to its effects on WS as well as its specific mode(s) of toxicity. Therefore, the goal of this study was to characterize molecular pathways of toxicity of SeM in WS and link these with specific physiological effects. Juvenile sturgeon were administered food spiked with 0, 5, 25, or 100 $\mu g \cdot g^{-1}$ dry mass of SeM for 72 days. After 10 days, a sub-sample was collected and liver samples from control- and medium-dosed fish were analyzed by use of Illumina sequence-by-synthesis technology to investigate transcriptional responses and identify physiological processes that might be adversely impacted by exposure to SeM. Investigation of transcriptional responses by use of RNA-seq indicated 376 transcripts were ≥ 2 fold up-regulated and 348 were ≥ 2 fold down-regulated, while 13,175 transcripts remained unchanged. Differentially expressed transcripts were analyzed through ClueGO, a Cytoscape plug-in, to determine gene ontology categories and their relationships. Significant changes were observed in genes involved with regulation of carbohydrate, lipid, cholesterol, amino acid and xenobiotic metabolism, as well as apoptosis, suggesting these pathways might play a role in mediating SeM toxicity in sturgeon. Further investigations are currently underway that link these changes at the molecular level with apical outcomes determined in a parallel study, the ultimate goal being to identify the molecular initiating events that are responsible for adverse outcomes of the exposure of WS to SeM.

Potential metabolic and cardiovascular effects of dietary selenomethionine exposure in adult zebrafish (*Danio rerio*) (PO)

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Selenium (Se) is an essential micronutrient involved in important metabolic functions for all vertebrate species. As Se is reported to have a narrow margin between deficiency and toxicity, there is growing concern surrounding the adverse effects of elevated Se exposure caused by anthropogenic activities. It is well known that oviparous vertebrate species, especially fish, are highly susceptible to elevated dietary Se

exposure. Recent studies have reported that elevated dietary exposure of fish to selenomethionine (Se-Met), the primary form of Se in the diet, can alter metabolic capacity, energy homeostasis, and swimming performance and can cause a greater incidence of early life stage deformities and mortality. This study aims to further investigate mechanisms of Se-Met toxicity, particularly potential underlying cardiovascular implications of chronic exposure to environmentally relevant concentrations of dietary Se-Met in adult zebrafish (*Danio rerio*). Adult zebrafish will be fed either control food or Se-Met spiked food with nominal concentrations of 10 and 30 $\mu g\ Se \cdot g^{-1}$ dry weight for 60 days at 5% body weight per day. Following exposure, high resolution B-mode and Doppler ultrasound will be used to characterize cardiac and vascular function. Metabolic endpoints will include whole body glycogen and triglyceride stores, and oxidative stress biomarkers (e.g., GSSG:GSH ratio). The results of this study will help further characterize sublethal physiological responses in fish inhabiting Se-contaminated aquatic ecosystems.

Assessing the fate and effect of dietary-Se on *Daphnia magna* using a modified two-generation life-cycle test (PO)

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Much attention has been given to the effects of selenium on fish, with relatively few studies conducted to assess the effects in invertebrates. This study assessed effects of dietary Se to the cladoceran *Daphnia magna* over two generations. An algal diet consisting of both *Chlorella* spp. and *Pseudokirchneriella subcapitata* was spiked in a dose-dependent manner with selenite over a 7-day growth period, resulting in a dietary-Se exposure of 5, 10, 20, 40 and 80 $mg \cdot kg^{-1}$ Se. First generation *D. magna* were fed over a 21-day period to assess survival and reproduction, offspring were collected and either exposed to dietary-Se or a control diet for a further 21 days. Tissue concentrations of both 1st and 2nd generation *D. magna* were assessed as well as visual inspection of the daphnids for any potential deformities. No effects on 1st or 2nd generation reproduction were observed in any of the dietary exposures. Survival, weight and length did not change significantly from controls. Tissue Se concentrations and trophic-transfer factors will also be presented.

Alternative Non-vertebrate Test Methods

An examination of selenium-cadmium interactions using primary cultures of rainbow trout (*Oncorhynchus mykiss*) hepatocytes (PL)

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The main objective of this study was to evaluate the ameliorative properties of selenium (Se) against cadmium (Cd)-induced oxidative stress in rainbow trout (*Oncorhynchus mykiss*) hepatocytes in primary culture. Cadmium (Cd) induces cytotoxicity by disruption of cellular oxidative homeostasis. On the other hand, selenium (Se) is an essential component of biological anti-oxidative machinery. However, Se can turn into a pro-oxidant beyond the threshold concentration. In this experiment, trout hepatocytes were exposed to 100 µM Cd, alone or in combination with different concentrations (25, 50, 100, 250 and 500 µM) of selenite or selenomethionine for 48 hours. It was observed that both forms of Se, up to a concentration of 100µM, significantly reduce Cd-induced cytotoxicity (measured as cell viability). Reduced cytotoxicity of Cd in the presence of Se was associated with reduced reactive oxygen species production, increased thiol status, and up-regulation of key anti-oxidative enzymes. Treatment with pharmacological anti-oxidants, such as TEMPO ((2,2,6,6-tetramethylpiperidin-1-yl)oxyl) and NAC (N-acetylcysteine), also reduced Cd-induced oxidative stress in trout hepatocytes, providing further evidence that Se likely ameliorates Cd toxicity via anti-oxidative mechanisms.

Development of sentinel species of benthic invertebrates for use in estuaries (PL)

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An effective environmental monitoring system requires current information regarding the physical properties of the environment and the physiological information of the ecosystem's inhabitants. Samples were collected in Saint John Harbour, NB, from 2011 to 2012, to measure sediment contamination and population characteristics of two benthic invertebrate species, the Atlantic nut clam, *Nucula proxima*, and the catworm, *Nephtys incisa*. The goals were, first, to characterize their seasonal and spatial variability, determine the ideal time and method of sampling, and generate up-to-date data for use as a baseline, and, second, to evaluate whether sediment contamination is impacting invertebrates within the harbour through comparing metal concentrations to growth and abundance of benthic infauna. Polycyclic aromatic hydrocarbons (PAHs) were low at all sites, with several compounds being below detection limits. Metal concentrations

were generally below CCME's interim sediment quality guidelines (ISQGs) for all metals except nickel, which was above guidelines at all sites. One site also exceeded ISQG for copper, zinc, and lead. *Nucula proxima* densities ranged from 311 ± 103 to 2782 ± 1533 clams·m^{-2} and were highly variable between sites but not seasons. *Nephtys incisa* densities were between 46 ± 22 and 292 ± 225 worms·m^{-2} and were not significantly different between sites or seasons. Measuring the impact of contamination on invertebrates proved difficult, as there was little variability in contaminants between sites, and major differences in sediment composition. Overall, sites showed little evidence of impacts from contamination.

Characterization of metallothionein in two ancient fishes exposed to metals: White sturgeon and lake sturgeon (PL)

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To date, all 24 species of sturgeons found in Europe, Asia, and North America are protected under the Convention on the International Trade of Endangered Species of Wild Fauna and Flora (CITES). Declines of sturgeon populations are thought to be the result of alterations to habitat, over harvest, predation by or competition from introduced species, exposure to pathogens, and pollution. Metals are one class of pollutants of concern to populations of sturgeons, with primary sources being past and present activities of mines, metallurgical facilities, pulp and paper mills, and other industrial and municipal input sources leading to the exposure of sturgeons to metals. White sturgeon (*Acipenser transmontanus*) are among the most sensitive species of fishes to copper (Cu), cadmium (Cd) and zinc (Zn), but there is no information about the sensitivity of lake sturgeon (*Acipenser fulvescens*) to these metals. To begin to elucidate the molecular mechanism(s) of sensitivity of sturgeons to metals, a cDNA encoding metallothionein (MT) was amplified from livers of white sturgeon (WS-MT) and lake sturgeon (LS-MT), and expression in response to Cu, Cd, or Zn was characterized in liver explants from each species. *In vitro* liver explants were chosen to address concerns of the large number of animals required by current toxicity testing approaches, in particular when working with species at risk such as sturgeon. The primary structure of WS-MT and LS-MT contained 20 cysteine residues, which is the same as MTs of teleost fishes. However, the primary structure of WS-MT and LS-MT contained 63 amino acids, which is longer than any MT identified in teleost fishes. Abundance of transcripts of WS-MT in explants exposed to 0.3, 3.0, 30, or 100 µg·L^{-1} of Cu was 1.7-, 1.7-, 2.1-, and 2.6-fold less than in controls, respectively. In contrast, abundances of transcripts of WS-MT were 3.3- and 2.4-fold greater in explants exposed to 30 µg·L^{-1} of Cd and 1000 µg·L^{-1} of Zn, respectively. Abundance of transcripts of LS-MT was not significantly different at any concentration of Cu, Cd, or Zn. MT is hypothesized to represent a critical mechanism for detoxification of metals. Results of this study suggest that sensitivity of sturgeons to exposure to Cu, Cd, or Zn is likely not a result of MT proteins that are inefficient or incapable of binding metals, but might be a result of the relatively lesser maximal response of MT to metals. The study also suggests lake sturgeon might be more sensitive than white sturgeon to metals.

Cross-species comparison of relative potencies and relative sensitivities of fishes to dibenzo-p-dioxins, dibenzofurans, and polychlorinated biphenyls *in vitro* (PL)

Bryanna Eisner[1], Jon Doering[1], Shawn Beitel[1], Steve Wiseman[1], Jason Raine[1] and Markus Hecker[1]
[1]University of Saskatchewan

Dioxin-like compounds (DLCs) of varying toxicities are found in complex mixtures. In order to enable streamlined risk assessment of DLCs, the toxic equivalency factor (TEF) approach was developed based on the potency of a DLC relative to the potency of the most toxic DLC. One limitation of this approach is uncertainty regarding differences in relative potency (ReP) among different species. RePs among fishes are limited relative to RePs for DLCs among birds and mammals, and TEFs for fishes are based entirely on the model test species, rainbow trout (*Oncorhynchus mykiss*). A liver explant assay was utilized to characterize species-specific responses with regard to up-regulation of CYP1A transcript following exposure to DLCs in rainbow trout, white sturgeon (*Acipenser transmontanus*), lake sturgeon (*Acipenser fulvescens*), and northern pike (*Esox lucius*). Differences in sensitivities were observed among species, with rainbow trout having the greatest sensitivity. RePs developed from liver explants of rainbow trout were comparable to RePs developed from embryos. RePs ranged by up to 40-fold among the species tested. To compare RePs among species, concentrations of DLCs in eggs in the Fraser River and Lake Ontario were used to calculate toxic equivalents (TEQs) using TEFs or TCDD equivalents (TCDD-EQs) using RePs. It was demonstrated that TEQs accurately represent TCDD-EQs for rainbow trout. TEQs underestimated TCDD-EQs for white sturgeon, lake sturgeon, and northern pike.

An animal testing strategy for industry: A Canadian perspective (PL)

Jonathan Naile[1], Sarah Hughes[1], Graham Whale[2], Charles Eadsforth[1] and Chantal Smulders[3]
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In 2011, Canada used over 2.6 million animals for testing of which 1.3 million were fish and, with expanding regulatory requirements, this amount is expected to increase. Although animal testing is important for hazard assessment of new products and effluents, there is increasing pressure to reduce, replace, and refine traditional animal testing methods wherever possible, while still meeting legal obligations and protecting the environment. This is due in part to concerns with animal welfare globally and achieving sustainable development goals. Many companies like Shell are committed to eliminating the need to do testing involving vertebrate animals, with the aim to

replace animal testing by suitable alternatives while ensuring that industry can continue to innovate, develop and maintain new and safe products and technologies. Testing with fish, either for product testing or whole effluent toxicity testing, is the largest category of vertebrate animal testing conducted by or on behalf of Shell or Shell joint ventures. In 2014, 63% of Shell's fish use in North America was for our Canadian facilities. Of this 51% was for required regulatory compliance testing and the rest was for testing related to anticipated future regulatory requirements. As part of Shell's commitment to eliminate animal testing, a long-term approach toward elimination of fish testing requirements has been adopted and is governed by the 3Rs: replacement, reduction, and refinement. An overview of Shell's approach to eliminating fish testing will be presented along with experiences with some of these alternative test methods, including a combination of *in silico*, *in vitro* and *in vivo* techniques. It may be that no single method or test by itself is sufficient to replace fish testing, but using a weight of evidence approach could help to reduce or eliminate fish testing.

Is something fishy with Environment Canada's adoption of animal alternatives? (PL)

Lisa Taylor[1] and Rick Scroggins[1]
[1]Environment Canada

For over 40 years, beginning with the Canadian *Fisheries Act* in 1972, ecotoxicity testing has been an integral component of environmental conservation and protection activities in Canada. This Act provided the legal basis for the protection of fish and fish habitat caused by pollution. Standardized methods were required for scientific rigor and defensibility of the data. An acute lethality test using rainbow trout (*Oncorhynchus mykiss*) was developed for the testing of industrial effluents and oil spill dispersants. Flash forward to 2015 and the same test is being used for the same reason, despite the pressure to reduce, replace, and refine vertebrate testing in environmental science. To date, twenty-four standard biological tests have been researched, peer reviewed, and published by Environment Canada (EC). The majority of these methods do not involve testing of vertebrate species (e.g., invertebrates, bacteria, plants and algae). But when fish are included in a test suite, it becomes the ideal battery for environmental protection encompassing "fish and fish habitat". Today, public opinion polls indicate a decline in support for animal experiments. That said, the regulators are hesitant to omit fish in compliance testing due their significance as a human food source, food source for top predators (e.g., birds, bears), entertainment-sports fishing/tourism, commercial fisheries, and enforcement-legal proceedings, even though alternative tests with relative sensitivities now exist. This presentation will answer the following questions: What efforts has Environment Canada taken in the past to address animal use in testing and alternative tests? And what are the hurdles to incorporating alternative methods into Canada's regulatory environment?

Exploring right hand coiled coil (RHCC) proteins as a matrix for a new aquatic passive sampling device for PAHs (PO)

Vince Palace[1], Jorg Stetefeld[2], Matthew McDougall[2], Mark Hanson[2] and Jennifer Leslie[1]
[1]Stantec Consulting Ltd., [2]University of Manitoba

Passive sampling devices (PSDs) are increasingly used to determine the bioavailability of contaminants in aquatic systems. PSDs accumulate contaminants similarly to the biota they are intended to model and are constructed from various adsorptive materials. They are retrieved easily, extracted inexpensively, and provide certainty of contaminant occurrence in the area of interest. Current PSDs have relatively long times associated with reaching equilibrium, which becomes problematic when volatiles are present. Right-handed coiled coil (RHCC) is a protein isolated from bacteria that reside in deep sea hydrothermal vents. The protein is thermally stable, resistant to a wide range of salinity, pH, and sulfide concentrations, and can be custom synthesized in the laboratory in a variety of polymeric forms. The binding of 16 priority polycyclic aromatic hydrocarbons (PAHs) to the RHCC protein was examined in a series of benchtop experiments. X-ray crystallography was used to demonstrate binding of each PAH in the internal cavity of the protein. These studies demonstrate the potential for RHCC proteins to be used as a new matrix for PSDs for modeling the bioavailability of PAHs.

Risk Assessment and Regulatory Toxicology

The case for deriving site-specific water quality objectives driven by science (PL)

Albert Shpyth[1], Don Hart[1] and Ron Nicholson[1]
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Methods for deriving site-specific water quality objectives (SSWQOs) have been available to Canadian ecotoxicologists (and others) for at least two decades (e.g., U.S. Environmental Protection Agency (USEPA) 1985, British Columbia Ministry of Environment 1997). However, not all regulatory jurisdictions in Canada encourage their use and instead rely on more generic guidelines for the protection of drinking water supplies, aquatic life and wildlife, agriculture, recreation and aesthetics, and to provide for industrial use of water resources. This paper explores whether SSWQOs should be allowed and, if so, what considerations they should entail. Supporting evidence that SSWQOs are science-based alternatives to generic objectives, and requirements for their development, will be presented. For example, our experience is that toxicity modifying factors are equally effective whether natural or anthropogenic, and should be considered in setting SSWQOs. We have also found that arbitrary safety factors have little basis in science. SSWQO protocols should allow recalculation of a minimal effect level, without a safety factor, consistent with Canadian Council of Ministers of the Environment (2007) and USEPA approaches. The field of environmental science and management has evolved since site-specific water quality objectives were first proposed for use in Canada, and consideration of environmental outcomes and cumulative effects requires the use of practical, science-based SSWQOs. With ever increasing demands on and competition for water resources, there is a need to be informed by biological effects data and factors that influence the bioavailability of constituents of potential concern, if we are to optimize the services derived from aquatic ecosystems.

Applicability of risk assessment and toxicology in regulating Alberta's Energy Resources (PL)

Mandy Dumanski[1]
[1]Alberta Energy Regulator

Energy regulation in Alberta spans more than 75 years and has evolved over time. This evolution continued in 2013 when the Alberta Energy Regulator (AER) became a new organization and began taking on regulatory functions related to energy development that were previously held by Alberta Environment and Sustainable Resource Development (ESRD). The AER is now the single regulator of ESRD Policy for energy development in Alberta. Regulation of energy resources covers the full life cycle of historically contaminated sites and proposed developments—from application and exploration to construction and development, and finally abandonment, remediation,

and reclamation. This talk will outline the policy (Tier 1 and 2 Soil and Groundwater Remediation Guidelines and Surface Water Quality Guidelines) provided by the Provincial Government against which contaminated sites are assessed and the applicability and limitations of risk assessment within this policy. The discussion will specifically address Alberta-based risk assessment principles, which include a tiered approach from simplistic pathway elimination and/or modifications to complex site-specific risk assessments, which incorporate toxicity testing programs and the development of site-specific remedial objectives. This dialogue will outline the decision making processes used by regulators to evaluate recently published receptor- and contaminant-specific toxicity data, and how this data may be incorporated into risk assessments at specific sites as well as in the derivation of Provincial Guidelines. Throughout the presentation, case studies presenting human and ecological risk assessments conducted on contaminated sites in Alberta will be used to illustrate the following key concepts: 1) defining closure within the regulatory environment, 2) outlining limitations of the risk assessment and potential factors which may limit closure options, 3) development of an appropriate conceptual site model in the end land use or post-closure scenario (i.e., single receptor exposure vs. multiple receptor exposure at a single trophic levels vs. complex food web exposure model with potential biomagnification of contaminants), 4) evaluating short- and long-term risk management or ongoing monitoring which may be required, and 5) the necessity of interpreting the results of the risk assessment with respect to environmental and human health implications.

Developing nitrogen species limits for a mine effluent discharged to a non-fish-bearing stream (PL)

Meghan Goertzen[1] and Robert Goldblatt[1]
[1]Lorax Environmental Services Ltd.

Nitrogen-based explosive use at mining operations can result in elevated levels of ammonia, nitrite, and nitrate in downstream aquatic systems that have the potential to cause toxic effects to certain biota. Although mine blasting residues yield variable mixtures of nitrogen (N) species, toxicity-based water quality guidelines are commonly generated from laboratory tests of single parameters. In this study, an integrated site-specific approach was used to develop effluent concentration limits for N species in water discharged from the underground workings of a high-elevation gold project in northern Canada. Limits were constrained to ensure underground effluents would meet the following criteria: 1) pass 48-hour *Daphnia magna* acute toxicity tests; 2) avoid causing acute toxicity to biota within the effluent mixing zone of the receiving stream; and 3) achieve year-round compliance with water quality guidelines and site-specific benchmarks at the point of complete mixing. In conjunction with a desktop literature assessment, standard dilution-series toxicity tests were conducted with site water using aquatic and benthic species representative of the receiving stream aquatic community. Test waters were spiked with ammonia, nitrite, and nitrate to create concentrations and ratios resembling mine discharge. An uncertainty factor was subsequently applied to the most sensitive toxicity endpoint to achieve a proposed effluent concentration limit, which was validated in a mass-balance loading model for the receiving stream under various monthly flow conditions. The results of this exercise support the use of a site-specific approach for setting discharge limits for complex effluents containing mixed N species.

Assessing our standards: The effect of temperature on toxicity of metal salts to six Canadian cold-water fish species (PL)

Dana Moore[1], David Poirier[2], Paul Sibley[3] and Keith Solomon[3]
[1]University of Manitoba, [2]Ontario Ministry of Environment and Climate Change,
[3]University of Guelph

Resource development in Canada's north has increased in recent years and has led to concerns about potential effects on aquatic biota, such as fish. To test the resiliency of the Environment Canada (EC) reference method used to prevent deleterious effects to cold-water fish species as mandated by the *Fisheries Act* of Canada, comparative acute toxicity testing of six industrial contaminant salts ($CaCl_2$, $MgCl_2$, KCl, NaCl, Na_2SO_4, and Mo_2O_4) was conducted using the reference species (rainbow trout (*Oncorhynchus mykiss*)) and five northern species of concern (arctic char (*Salvelinus alpinus*), lake trout (*Salvelinus namaycush*), lake whitefish (*Coregonus clupeaformis*), round whitefish (*Prosopium cylindraceum*), and arctic grayling (*Thymallus arcticus*)) at three temperatures (5, 10, and 15°C). The ability of the reference species used by EC to protect other cold-water fish was assessed by comparing the 96-hour rainbow trout LC_{50} for each salt at 15°C to the 96-hour LC_{50} for each non-standard species at 5, 10, and 15°C. Reference tests were conducted in-lab using the conditions prescribed by the EC test method for monitoring of industrial effluent using rainbow trout; novel tests were conducted by reproducing the EC assay while varying the species and temperatures tested so as to characterize the influence of these parameters on the observed toxicity. Comparison between the reference and novel measures revealed that fish exhibited significantly greater sensitivity to the salts tested than did rainbow trout at 15°C in 32% of the novel assays and in 69% of the novel chloride salt assays; with only Na_2SO_4 confirming rainbow trout as a protective surrogate species for cold-water fish. This differential sensitivity between standard surrogate species and the native species they aim to protect may have profound impacts in northern locales—such as the Snap Lake Diamond Mine in the Northwest Territories—where industrial monitoring programs have been designed based on the surrogate species, rainbow trout, and exceedance of TDS limits in effluent are common.

Ecological risk assessment at the former Cutler Acid Plant, Ontario: Approaches for evaluating risks to aquatic receptors in lake, stream, ditches and marshy areas (PL)

Heather Fenton[1], Nicole MacDonald[2], Kerrie Serben[1], Michael Z'Graggen[1], Roy Angelow[3], Sue-Jin An[4] and Barbara Slezak[4]

[1]Golder Associates Ltd., [2]CLAW Environmental Services Inc., [3]Aboriginal Affairs and Northern Development Canada, [4]Public Works and Government Services Canada

The 40-hectare site of the former Cutler Acid Plant is located within a First Nation community along the shore of Lake Huron (Aird Bay). From 1957 to 1963 the plant produced sulfuric acid for uranium extraction at mines in northern Ontario. Plant operations, including stockpiling and processing of sulfide minerals, a tailings pond, and product storage, imparted acidic and metal solutions to the subsurface and impacts to on-site watercourses and the lake. Historic remediation efforts included the removal of stockpiles, tailings, and visually contaminated soil to the water table, lime amendment at the water table, and importation of clean fill. In addition to low soil pH, contaminants of concern remaining on site post-remediation include: sulfur, sulfate, chloride, and several metals, metalloids, and non-metals including selenium. An aquatic ecological risk assessment (ERA) was completed using a weight-of-evidence approach as described by the Federal Contaminated Sites Action Plan (FCSAP) Ecological Risk Assessment Guidance. A key challenge of the aquatic ERA was consideration of three different aquatic habitats with varying sets of aquatic receptors and operable exposure pathways, including the near-shore area of the lake and watercourses on Site that include a fish-bearing stream (West Stream) and non-fish-bearing ditches and marshy areas. Within the impacted near-shore area of the lake, habitat characteristics and characterization of background conditions were the key components of the risk assessment that concluded that risks were acceptable. Within the on-site watercourses, risks were evaluated based on several lines of evidence, including: assessment of macrophyte communities and health, assessment of benthic invertebrate communities, assessment of diversity and abundance of fish species, surface water toxicity testing to evaluate site-specific effects on growth of *Pseudokirchneriella subcapitata* (phytoplankton) and on survival and reproduction of *Ceriodaphnia dubia* (pelagic invertebrates), and sediment toxicity testing to evaluate site-specific effects on survival and growth of *Hyalella azteca* and *Chironomus dilutus* (benthic invertebrates). For the West Stream, unacceptable risks were identified and site-specific target levels were derived for iron and cobalt in surface water, and pH, sulfur, sulfate, copper, and selenium in sediment. For ditches and marshy areas, determined not to be fish habitat, unacceptable risks were identified and it was concluded that blocking of exposure pathways by eliminating these watercourses would not likely affect ecological receptors at the community/population level.

Ecological risk assessment at the former Cutler Acid Plant, Ontario: Assessing risks to ground-dwelling invertebrates in low-pH, metal-contaminated soil using a weight-of-evidence approach (PL)

Heather Fenton[1], Roy Angelow[2], Tammie Morgan-Gray[1], Nicole MacDonald[3], Michael Z'Graggen[1], Sue-Jin An[4] and Barbara Slezak[4]

[1]Golder Associates Ltd., [2]Aboriginal Affairs and Northern Development Canada, [3]CLAW Environmental Services Inc., [4]Public Works and Government Services Canada

The 40-hectare site of the former Cutler Acid Plant is located within a First Nation community along the shore of Lake Huron (Aird Bay). From 1957 to 1963, the plant produced sulfuric acid for uranium extraction at mines in northern Ontario. Plant operations, including stockpiling and processing of sulfide minerals, a tailings pond, and product storage imparted acidic and metal solutions to the subsurface. Historic remediation efforts included the removal of stockpiles, tailings, and visually contaminated soil to the water table, lime amendment at the water table, and importation of clean fill. In addition to low soil pH, contaminants of concern remaining on site post-remediation include: sulfur, sulfate, chloride, selenium, and metals. A terrestrial ecological risk assessment was completed using a weight-of-evidence approach as described by the Federal Contaminated Sites Action Plan (FCSAP) Ecological Risk Assessment Guidance. To evaluate risks to ground-dwelling invertebrates, three lines of evidence (LOE) were used: comparison of contaminant concentrations to literature-based toxicity benchmarks (LOE 1); assessment of habitat suitability and presence/absence and abundance of earthworms and other ground-dwelling invertebrates on site (LOE 2); and 3) soil toxicity testing to assess site-specific effects of selenium on survival, growth, and reproduction of *Eisenia andrei* (earthworm) (LOE 3). Based on LOE 1, potentially unacceptable risks were identified for soil pH, sulfate, and selenium. The results of LOE 2 indicated the site provides poor quality habitat for ground-dwelling invertebrate communities. LOE 2 also indicated that communities may be negatively affected by selenium concentrations above the toxicological benchmark of 4.1 $\mu g \cdot g^{-1}$, but was inconclusive with respect to risks associated with other contaminants. The results of earthworm toxicity testing (LOE 3) indicated that selenium concentrations of up to 50 $\mu g \cdot g^{-1}$ do not pose unacceptable risks to ground-dwelling soil invertebrate communities on Site. Based on consideration of all LOE, site-specific target levels were derived for selenium, soil pH, and sulfate.

Using saturation kinetics-based models to predict *Hyalella azteca* mortality in chronic zinc exposures (PL)

Lesley Milne Wilkinson[1], Warren Norwood[2] and D. George Dixon[1]

[1]University of Waterloo, [2]Environment Canada

Mechanistic models that use saturation kinetics have been developed over the past few decades with the goal of predicting site-specific metal toxicity in aquatic

systems. Mortality models with Michaelis-Menton kinetics-type parameters can predict mortality in *Hyalella azteca* for a number of different metals based on water or tissue concentrations. If lethal tissue concentrations remain constant in different water chemistry conditions, measuring site-specific water chemistry may not be required to predict toxicity. Such measurements are necessary for other mechanistic models, like the Biotic Ligand Model (BLM). Experiments were conducted in different water chemistry conditions (varying pH, alkalinity, and dissolved organic carbon) to determine effects of a waterborne concentration series of zinc chloride (ZnCl2) on mortality, growth, and bioaccumulation in chronic (28-day) exposures. These tests were conducted in moderately soft water using the epibenthic amphipod, *H. azteca*. In previous studies by this group, *H. azteca* were exposed to zinc in moderately hard water under fixed water chemistry conditions in chronic exposures. Higher concentrations of dissolved organic carbon, increased alkalinity, and increased hardness decreased mortality, which is what is predicted by BLMs. Objectives of this work are: (1) to determine if exposure water chemistry impacts the lethal tissue concentration and (2) to modify saturation mortality models to reflect site-specific water chemistry, if necessary.

Large-scale risk assessment of organic chemicals in river ecosystems: A European perspective (PL)

Egina Malaj[1], Peter von der Ohe[1], Matthias Grote[2], Werner Brack[1] and Ralf Schäfer[1]

[1]Helmholtz Centre for Environmental Research-UFZ, [2]Electricité de France

The increasing anthropogenic demand for organic chemicals has created large environmental problems with repercussions for the health of the environment, especially of aquatic ecosystems. While the adverse effects of organic chemicals on freshwater ecosystems have been extensively demonstrated locally or regionally, their overall relevance regarding large spatial scales remains unknown. Large-scale analyses are crucial not only to identify chemical hot spots, but also to direct the protection and rehabilitation efforts for aquatic ecosystems as a whole. The current research sought to investigate the widespread risk from organic chemicals in river ecosystems on a continental scale. The risk for acute lethal and chronic long-term effects on fish, invertebrate, and algae species was investigated in 4,000 monitoring sites distributed throughout 27 European countries and 91 respective river basins. Furthermore, the ecotoxicologically-relevant chemicals and their sources were identified. Organic chemicals were likely to exert acute lethal and chronic long-term effects on fish, invertebrate, or algae species in 14% and 42% of the sites, respectively. Of the 223 chemicals monitored, pesticides, tributyltin, polycyclic aromatic hydrocarbons, and brominated flame-retardants were the major contributors to the chemical risk. There was a significant difference in the chemical risk between sites with intensive agriculture and/or urban practices (more than 50% land use) and those with natural vegetation (more than 80% land use; $p<0.05$). The risk of acute and chronic effects increased with the number of ecotoxicologically-relevant chemicals analyzed at each site. Therefore, the chemical risk is likely underestimated for sites that measure only a few relevant compounds. Other reasons which might have underestimated the chemical risks are (i) the limited number of chemicals that were routinely analyzed in monitoring programs, (ii) the high limits of quantification, (iii) the presence of mixtures, or (iv) the potential for sublethal effects which are not covered by direct toxicity. Overall, the results indicate that chemical pollution is a continental problem and as such requires large-scale integrated solutions.

The EU project SOLUTIONS: Challenges and responses for present and future emerging pollutants in land and water resources management (PL)

Henner Hollert[1] and Werner Brack[2]
[1]RWTH Aachen University, [2]Helmholtz Centre for Environmental Research

SOLUTIONS (2013 to 2018) is a European Union Seventh Framework Programme Project (EU-FP7). The project aims to deliver a conceptual framework to support the evidence-based development of environmental policies with regard to water quality. SOLUTIONS searches for new and improved tools, models, and methods to support decisions in environmental and water policies. The overall goal of the FP7 Integrated Project SOLUTIONS is to produce consistent solutions for the large number of legacy, present and future chemicals posing a risk to European water resources with respect to ecosystems and human health. To this end, SOLUTIONS (1) develops a consistent conceptual framework for the assessment, prioritization and abatement of pollutants and mixtures thereof to protect European water resources and to minimize ecological and human health risks; (2) delivers efficient tools for the identification of substances and mixtures posing risks by developing a new generation of monitoring approaches and tools for the early detection and identification of harmful substances. At the same time, SOLUTIONS (3) provides improved understanding and capacity for exposure, effect, and risk modelling, compiling a full chain of conceptually integrated models and databases accessible via a user-friendly computer tool to support decisions in environmental and water policies; and (4) demonstrates the added value of the new generation of tools in trans-European case studies in the Danube, Rhine, and rivers of the Iberian Peninsula, with links to existing monitoring programs such as the Joint Danube Survey. For further information: Brack *et al.* (2015): The SOLUTIONS project: Challenges and responses for present and future emerging pollutants in land and water resources management. Science of the Total Environment 503, 22-31. The SOLUTIONS project is supported by the European Union Seventh Framework Programme (FP7-ENV-2013-two-stage Collaborative project) under grant agreement no. 603437.

Is received dose from ingested soil independent of soil PAH concentrations: Animal model results (PO)

Rachel Peters[1], Mark Wickstrom[1] and Steven Siciliano[1]
[1]University of Saskatchewan

It is accepted that polycyclic aromatic hydrocarbon (PAH) bioavailability from ingested soils will vary between soils; however, the nature of this variation is not well characterized. Here, we used the juvenile swine model to link external exposure to internal benzo[a]pyrene (BaP) and anthracene exposure following oral PAH ingestion of 27 different real-world soils, soots, or spiked artificial soils. Internal exposure of BaP and anthracene, represented by area under the plasma-time curve (AUC), did not correlate to soil concentration in real-world soils, but did correlate in spiked artificial soil. Point of departure (POD) modeling identified soil PAH concentrations greater than 1,900 $mg \cdot kg^{-1}$ as the concentration where internal exposures become proportional to external doses. BaP and anthracene internal exposure below 1,900 $mg \cdot kg^{-1}$ averaged 21% of external exposure. Weak correlations between soil:simulated gastrointestinal fluid PAH partitioning and AUC values indicate that desorption from soil does not play a large role in influencing internal exposure of PAHs. We propose four PAH risk assessment options: (i) assume 100% bioavailability, (ii) assume constant internal exposure below 1,900 $mg \cdot kg^{-1}$, (iii) assume less than 100%, e.g., 21%, bioavailability below 1,900 $mg \cdot kg^{-1}$, or (iv) model internal exposure through AUC versus soil characteristic relationships. In our opinion, our data best supports option (ii) because we could not detect an increase in AUC with increasing soil concentrations and our best efforts at (iv) do not robustly predict uptake of different PAHs.

Herbicides in Manitoba: Estimating environmental exposure and improving aquatic monitoring in the province (PO)

Dana Moore[1], Charles Wong[2] and Mark Hanson[1]
[1]University of Manitoba, [2]University of Winnipeg

Since 1995, the Province of Manitoba's Pesticide Monitoring Program has amassed data for 89 compounds from more than 200 unique sampling sites. Many of these pesticides can have significant ecological effects on fish populations—both directly (e.g., insecticides through mortality) and indirectly (e.g., herbicides through changes in primary productivity)—and include those of public and regulatory concern, such as the neonicotinoid insecticides and the corn herbicide atrazine. Working together with Manitoba Conservation, this study provides a probabilistic ecological risk assessment for pesticides based on this provincial data cache, in combination with available literature and data from other monitoring programs (i.e., Environment Canada). Using probabilistic techniques, the assessment aims to predict the likelihood of observing a pesticide through space and time, as well as the likelihood of exceeding water quality guidelines. Preliminary analyses indicate that the times, locations, and compounds monitored in the program could be improved to create a more robust data set at a

reduced cost. Focusing on the 47 herbicides monitored—using the example compounds of atrazine, glyphosate, and 2,4-D—the presentation will address how this regional program assessment will help to identify the location of pesticide hotspots; predict future pesticide levels based on application rate increases or decreases; characterize trends between pesticide application rates or sites and the subsequent environmental concentrations; and make recommendations for a renewed monitoring initiative in the province. This approach has been adopted globally, but has yet to be employed in Manitoba.

Sulfonamide antibiotic transformation products in wastewater effluent dominated streams pose little risk of toxicity to aquatic organisms (PO)

Chelsea Lobson[1] and Mark Hanson[1]
[1]University of Manitoba

Human-use antibiotics are released into aquatic ecosystems with municipal wastewater effluents. The sulfonamides are a class of antibiotics commonly detected in surface waters globally as a result of these releases. Along with the parent compounds, transformation products (TPs) formed through various biotic and abiotic processes are also released; however, our understanding of TP occurrence, fate, and ecological risk is much less certain. The goal of this exercise was to predict which sulfonamide TPs may have direct toxicological effects at concentrations currently found in the environment. Relevant TPs formed by photolysis, hydrolysis, and biological degradation were determined by the literature, and University of Minnesota's Biodegradation Database Pathway Prediction System and Ecological Structure Activity Relationships (ECOSAR) predictive model were used to model toxicity for a variety of acute and chronic endpoints. Exposure distributions were constructed for measured average and maximum effluent concentrations for all sulfonamides examined, and assuming a 1:1 parent to TP production ratio. For example, the greatest measured environmental concentration for the commonly used sulfonamide antibiotic sulfamethoxazole was 8.71 $\mu g \cdot L^{-1}$. For sulfamethoxazole, ECOSAR endpoints had hazard quotients above one. However, the greatest probability of exceedance was 0.26% for a chronic endpoint (daphnid) exposed to aniline, and only two endpoints had probability of exceedances above 0.001%, suggesting there is little to no risk of direct toxicity associated with these TPs at current environmental concentrations. Overall, we recommend using this ecological risk assessment approach to identify data gaps for TPs, specifically for targeted toxicity testing.

New Substances Program tools used to assess the quality of test data during ecological risk assessment (PO)

Cristina Inglis[1]
[1]Environment Canada

One of the challenges of ecological risk assessment of substances is to ensure that submitted test data are reliable and relevant. Environment Canada's New Substances Evaluation Section uses specialized tools to assess the inherent quality of ecotoxicity test data as well as the appropriateness of test results for a particular hazard identification or risk characterization. This poster will describe elements of ecotoxicity test data that contribute to the overall validity and acceptability of a test and will highlight the benefits of using sound ecotoxicity principles during data generation. In addition, this poster will illustrate how advanced planning and a full understanding of the properties of a test substance can allow the assessment to proceed without delay.

Ecological screening assessment of substances in the "Certain Organic Flame Retardants" grouping under the Government of Canada's Chemical Management Plan (PO)

Jenny Ferone[1]**, John Pasternak**[1]**, Michael Beking**[1]**, Vincent Dorais**[1]**, Barbara Elliott**[1]**, Magdalena Jagla**[1]**, Jen-ni Kuo**[1] **and Kaiqi Yao**[1]
[1]Environment Canada

Under the Substance Groupings Initiative of the Chemicals Management Plan (CMP), the Government of Canada is assessing and will manage, where appropriate, the human health and ecological risks associated with nine groups of substances. These groups of substances are being assessed under the provisions of the *Canadian Environmental Protection Act, 1999* (CEPA 1999). One group of substances included in this initiative is the "Certain Organic Flame Retardants (OFR) Substance Grouping", composed of ten substances having a similar function (i.e., the application to materials to slow the ignition and spread of fire). The substance grouping considers OFRs with potential exposure to humans, that are used in high volume and that demonstrate potential persistence and inherent toxicity to nonhuman organisms. The OFR ecological risk assessments include a weight of evidence analysis which may consider, for instance, physical-chemical properties, sources and releases, chemical fate, persistence, bioaccumulation, long range transport, ecotoxicity, temporal trends, geographical distribution and exposure levels in Canada. Assessments are subject to external science peer reviews and public consultation prior to finalization. Draft technical approaches related to the ecological assessment of the OFR Substances will be presented. One particular focus is a draft approach to estimate release of the OFR Decabromodiphenyl ethane (DBDPE) to domestic laundry wastewater, as a source of OFRs to the aquatic environment. The poster will also consider other lines of evidence relevant to the assessment of DBDPE, such as physical and chemical properties and chemical

transformation. The draft assessments will be published for a 60-day public comment period in late 2015 on the Chemical Substances Website. Comments from the public will be considered for the final assessments to be published in 2016.

Behavioural Ecotoxicology

Use of behavioural endpoints in developing U.S. Environmental Protection Agency Water Quality Criteria: Yes, no, or maybe so? (PL)

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Historically, behavioural endpoints have not been used in the derivation of U.S. Environmental Protection Agency (USEPA) water quality criteria. USEPA criteria are intended to protect aquatic communities, and are derived by extrapolating organism-level effects on survival, reproduction, and growth to concentrations that are protective of 95% of aquatic genera in a community. Behavioural endpoints also operate at the organism level of biological organization, and thus have the potential to support criteria development. But to date, with only a few exceptions, behavioural data has not been considered for use in criteria development. The reasons for this include the lack of standardized behavioural test procedures, test endpoints, and exposure durations; the reversibility of many adverse behavioural effects if exposure is reduced or eliminated; and few observations of behavioural toxicity in field situations which could be used to confirm laboratory observations. In the USEPA's newer criteria documents, behavioural endpoints are increasingly discussed and summarized, consistent with the recognition that behavioural endpoints have direct ecological relevance. Behavioural endpoints are currently being used to evaluate whether existing USEPA water quality criteria are protective of threatened and endangered species within the U.S. But before behavioural endpoints will be routinely included in the derivation of new or the update of existing USEPA water quality criteria, the development of standardized behavioural test endpoints, test methodologies, and exposure durations will need to take place.

The role of water hardness in physiology and behaviour of freshwater organisms (PL)

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Effluent discharge resulting from anthropogenic activities (e.g., mining activities) can be a cause for an appreciable increase in water hardness cations (calcium and magnesium ions) in receiving waters. Contrary to this, some aquatic systems are witnessing a decline in calcium concentrations (e.g., lakes in eastern Canada, resulting in lower water hardness). Scientific evidence suggests that increasing water hardness ameliorates metal toxicity in aquatic organisms. Also, studies have shown that declining calcium concentrations can change species composition by replacement of a keystone species with its competitor (e.g., *Holopedium glacialis* replacing *Daphnia magna*). Calcium and/or magnesium ions can influence various physiological processes such as ion-regulation, gaseous exchange, reproduction, or endocrine function in aquatic organisms. Changes in such cation concentrations can cause physiological disruptions having the potential to cause toxic (lethal or sublethal) effects in aquatic organisms. Similarly, changing calcium concentrations can alter behaviour (e.g., predator-prey interactions between *Chaoborus americanus* and *Daphnia pulex*) in aquatic systems. Assessment of behavioural changes can link physiological functions with ecological processes in aquatic organisms. Behavioural studies can provide a meaningful assessment of toxicological risk. Thus behavioural toxicity testing can help understanding of how changes in animal behaviours (e.g., activity) can relate to broader ecological concerns such as survival, health and fitness. Not much is known about the effects of changing water hardness concentrations—especially extreme changes—in freshwater organisms. Freshwater systems witnessing hardness alterations due to natural or anthropogenic causes could have varying effects on inhabiting species such as unicellular algae all the way up to a predatory fish in the food chain. We are currently investigating lethal and sublethal effects (e.g., swimming performance, behaviour, physiological stress) of changing water hardness across a wide range of concentrations (0, 50, 165, 300, 600, 1200 $mg{\cdot}L^{-1}$ as $CaCO_3$) at a 1:1 Ca:Mg ratios in multiple freshwater species such as algae (*Raphidocelis subcapitata*), amphipod crustaceans (*Hyalella azteca*), and salmonid and non-salmonid fishes such as rainbow trout (*Oncorhynchus mykiss*) and fathead minnows (*Pimephales promelas*). Our findings from a fathead minnow hardness switch study suggest that a water hardness range of 50-600 $mg{\cdot}L^{-1}$ as $CaCO_3$ was not sufficient to cause mortality, impaired swimming performance, or impaired physiological stress response. Findings of this multi-species water hardness study will play a role in linking physiological and behavioural changes with the goal of establishing criteria for developing behavioural testing procedures for regulatory testing purposes.

Assessing "sublethal" effects of acidification in freshwater habitats: Lessons from Atlantic salmon in the Little Southwest Miramichi River (PL)

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Within aquatic ecosystems, a wide range of taxonomically diverse prey species rely on chemosensory information to assess local predation threats. Perhaps the most widely studied form of chemosensory information are the so-called "damage-released chemical alarm cues". Access to these alarm cues is known to provide valuable information about the form and intensity of risks and allows prey to balance the conflicting demands of detection and avoidance of predation threats and the need to forage, court and/or defend territories. While a growing body of research demonstrates that the detection of chemosensory information may be impaired as a result of anthropogenic contaminants, far less is known about the ecological effects of sublethal exposures. One such contaminant is stream-water acidification. Laboratory and field trials demonstrate that under weakly acidic conditions (~pH 6.0-6.4), prey fishes do not respond to damage-release chemical alarm cues. But what are the ecological consequences of this demonstrated "loss of function" of such a critically important source of chemosensory information? Here, we will provide a summary of our long-term research programme aimed at assessing the potential direct and indirect impacts of weakly acidic environments on the chemically-mediated behaviour of juvenile Atlantic salmon under fully natural conditions. We will discuss the potential implications our results may have for future stream restoration and management practices.

The antidepressant venlafaxine is a neuroendocrine disruptor and affects stress response and behaviour in fish (PL)

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Venlafaxine, a serotonin-norepinephrine reuptake inhibitor, is a widely prescribed antidepressant drug routinely detected in the aquatic environment. However, little is known about its impact on the physiology of non-target organisms. We have been investigating whether venlafaxine perturbation of brain monoaminergic levels may be a mechanism leading to neuroendocrine disruption in fish. We utilized stress response, feeding and behavioural responses as markers of performance dysfunction associated with venlafaxine exposure in rainbow trout (*Oncorhynchus mykiss*). Exposure of fish to waterborne venlafaxine (0.2 and 1.0 microgram per litre) for 7 days elevated norepinephrine, serotonin and dopamine levels in the brain in a region-specific manner. The drug treatment impacted feeding, but did not affect the establishment of social hierarchies. There was a clear effect of venlafaxine on the cortisol response to a physical and a social stressor in trout. The results suggest that the mode of action of venlafaxine may involve disruption of each target tissue involved in the HPI axis functioning. Specifically, *in vitro* mechanistic studies suggest that hypothalamus functioning is disrupted by venlafaxine and this may involve altered serotonin signalling. The molecular responses observed with venlafaxine in the hypothalamus include changes in mRNA levels of key genes involved in appetite regulation and stress response, including corticotropin releasing factor (CRF) and neuropeptide Y (NPY). At the pituitary level, venlafaxine impairs adrenocorticotropic hormone (ACTH) production, and this involves disruption of corticotropin releasing factor-receptor type 1 (CRF-R1), which is a key sensor for CRF stimulation. Complementary studies using zebrafish reveal for the first time that venlafaxine exposure may also affect neurogenesis. Overall, at environmentally-realistic levels venlafaxine is a neuroendocrine disruptor in fish and affects stress response and feeding behaviour in fish. The mode of action of the drug may involve altered serotonin signaling and/or impact on neurogenesis.

Local adaptation of finescale dace (*Phoxinus neogaeus*) olfaction, behaviour, and swim physiology post-exposure to natural and anthropogenic sources of bituminous toxicants (PL)

Sylvia Chow[1] and Greg Pyle[1]
[1]University of Lethbridge

Deposits of bitumen-rich oil sands are found in three major reserves located in northern Alberta's Peace River, Cold Lake, and Athabasca oil sands areas. The majority of surface-mineable oil sands deposits are located in the Athabasca oil sands area, the largest of the three deposits. Surficial oil sands are found within the lower Athabasca River basin where toxicants related to these deposits may enter watersheds through lotic erosion or oil sands mining or processing, natural and anthropogenic sources, respectively. Aquatic organisms found within this basin may be exposed to either or both sources of bituminous oil sands xenobiotics. When looking at smaller primary consuming cyprinids such as finescale dace (*Phoxinus neogaeus*), predator avoidance is an important factor to consider when looking at aquatic ecotoxicology, especially within such a large basin. In order to examine potential adaptive changes in response to anthropogenic and natural sources of bituminous toxicants, a reciprocal cross transplant of finescale dace has been completed. Transplant occurred among tributaries located downstream, and upstream of oil sands industry and within a tributary containing natural sources of bitumen. Before, during and after transplant exposure, fish olfaction, behaviour and swim physiology were examined. Olfaction was examined at the cellular level by examining responses of fish to conspecific cues using electroolfactography. By conducting electrophysiological measurements of electron transduction changes at the olfactory epithelium, response of the fish to a conspecific cue can be recorded as a negative potential change across the cell membrane. In order to illustrate this cellular-level response to conspecific cues at the organismal level, a behavioural bioassay is performed using an I-maze behaviour trough. Cue and control water are administered at either ends of the I-maze trough and fish observations are completed. As the ability of a fish to avoid a predation event is governed by a fish to escape the event, physiology related to the ability to swim has been examined as well. Physiological changes with respect to swimming abilities were determined by measuring respiration and swim tests. Respiration was measured as oxygen levels before and after testing. Swimming abilities were measured using repeat constant acceleration swimming performance test or U_{MAX}, which illustrates the ability of a small cyprinid to evade predation. When smaller fish are exposed to different xenobiotic regimes, adaptive changes may affect the surrounding ecosystems as cyprinids provide sustenance for piscivorous secondary consumers found in both the aquatic and terrestrial ecosystems. Adaptive changes in finescale dace can therefore help elucidate the environmental risks of anthropogenic and natural sources of bituminous oil sands in the lower Athabasca River basin.

The impact of diluted bitumen and conventional crude oil exposure on basal activity and visual learning behaviour in zebrafish (*Danio rerio*) (PL)

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The oil sands are the third largest proven oil reserve in the world and will bring countless jobs and revenue to the province of Alberta and the Canadian economy (Alberta Energy, 2014). The bitumen produced in the oil sands differs greatly in chemical composition to conventional crude oil, and to facilitate pipeline transport the bitumen has to be diluted with natural gas condensates to create diluted bitumen or "dilbit". The impacts of dilbit exposure on aquatic animals have not been well studied to date, despite the possibilities of accidental spills. In this study we examined the effects of crude oil and dilbit exposure on the swimming activity and visual learning behaviour of larval zebrafish (*Danio rerio*) 7 days post-fertilization (dpf). Embryos were exposed to water accommodated fractions (WAFs) of crude oil for a 7-day period and then video imaging software was used to measure the maximum velocity, distance travelled and time spent in the border vs. the center of their enclosures (24 well plates) over a 10-minute period. To test habituation to a visual simulation of a novel object, 7 dpf embryos were exposed to a pre-recorded video that repeatedly exposed embryos to a moving image. Data thus far suggest that exposure to high but not low concentration WAFs affects searching behaviour or anxiety, as exposed fish did not tend to leave the center of the well despite having similar swimming activities. Whole organism sub lethal endpoints, like basal activity and visual learning behaviour in zebrafish, could provide subtle cues as to the impact oil exposure has on fish fitness.

The role of suspended particulate matter on the toxicity of oil sands process-affected water on feeding behaviour of *Daphnia magna* (PL)

Ebrahim Lari[1], Effat Mohaddes[1] and Greg Pyle[1]
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Oil sands process-affected water (OSPW), which is produced as a byproduct of the surface-mining oil sands industry in northeastern Alberta, is currently being stored in massive on-site tailing ponds. Responsible remediation and ultimate release of OSPW to the natural environment is a concern to both oil-sand companies and regulators. Any release of OSPW, treated or otherwise, would need to minimize the impact to downstream ecosystems. In our previous studies we showed that OSPW decreases the feeding rate of *Daphnia magna*. We hypothesized that *Daphnia* consuming suspended inorganic particulate matter (SPM) in OSPW while they are filtering food, is one of the causes of the observed reduction in their feeding rate. To test this hypothesis we ran *Daphnia* feeding rate experiment with 3 substances (total OSPW, filtered OSPW, and glacier clay solution). In each experiment we examined the feeding behaviour of one-week-old *Daphnia* to each of the OSPWs. We exposed *Daphnia* to a dilution series (0, 1.25, 2.5, 5, 10 and 20%) of each substance, fed them similar quantities of

Pseudokirchneriella subcapitata, and gave them 24 hours (in darkness) to graze. After 24 hours, we determined the *Daphnia* food consumption by evaluating how much algae remained. We determined *Daphnia's* particulate matter consumption by measuring aluminum (Al) and silica (Si) content (as indicators of SPM) of test animals using ICP-MS. The results of the feeding rate test showed that both filtered and unfiltered OSPW reduce the feeding rate of *Daphnia*. Results from ICP-MS measuring the Al and Si content of the test animals' bodies showed increasing concentration of Al and Si by increasing the concentration of unfiltered OSPW and glacier clay solution, while no significant increase of these elements was observed in *Daphnia* exposed to filtered OSPW. Our results show that SPM plays a role in decreasing the feeding rate caused by OSPW, but there are other factors that take part in the toxicity of OSPW that need to be explored.

Behavioural methods in aquatic toxicology (PL)

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Behavioural responses are important to survival because they are necessary to perform essential life functions such as habitat selection, competition, predator avoidance, prey selection and reproduction. Thus, behavioural investigations are particularly pertinent to assessing the effects of environmental contaminants. Contaminants affect virtually every aspect of behaviour in aquatic organisms. The accumulated evidence indicates that certain behavioural functions are objective, well defined, readily quantifiable endpoints that are sensitive to sublethal contaminant exposure and are ecologically relevant. In addition, behaviour can regulate the extent of contaminant exposure experienced by the organism through contact with its environment and diet. The inclusion of behavioural test methods in toxicity evaluations often increases the sensitivity, ecological relevance and predictive capability of contaminant assessments. Observations of behaviour also provide a unique toxicological perspective since behaviour links the biochemical and ecological consequences of environmental contamination. Certain behavioural indices have been used to measure contaminant effects and injury, to provide diagnostic identification of contaminants, and to predict ecological impact, enabling the evaluation of risks and establishing critical thresholds. Behavioural testing achieved legal status in the U.S. with the formulation of Natural Resource Damage Assessment procedures for assessing damages to natural resources from discharges of hazardous substances, and is also included in assessments conducted under the *Oil Pollution Act*. Efforts to standardize behavioural methods are proceeding through an American Society for Testing and Materials (ASTM) Committee E47 in an effort to increase the utilization of behavioural data in the assessment of environmental contamination.

Acute and chronic exposure to treated wastewater effluent affects movement and aggression in round goby (PL)

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The effect of wastewater effluent on fish physiology, specifically reproductive physiology, has received much attention over the last 25 years. However, less is known about how treated effluent affects fish behaviours, such as aggression, that are important for survival and fitness. Here, we exposed male and female round goby (*Neogobius melanostomus*) to treated wastewater effluent collected from a Hamilton Harbour, Lake Ontario (Canada) wastewater treatment facility. Fish were exposed in the laboratory to 0, 25, 50, or 100% effluent acutely for 3 days, or chronically for 28 days. After exposures, we assessed movement and aggression in the laboratory. We found that fish were less active after an acute exposure, and were less aggressive only after the chronic exposure. We discuss our results in relation to tissue burdens of pharmaceutical compounds, and relate our findings to potential impacts on fish fitness in the wild.

Multi-Generational Toxicology and Epigenetics

Larval exposure to fluoxetine suppresses the stress response in adult zebrafish across one generation (PL)

Marilyn Vera Chang[1], Thomas Moon[1] and Vance Trudeau[1]
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The ubiquitous presence of a vast array of pharmaceuticals and other chemicals in the aquatic environment has the potential to disrupt the endocrine system of aquatic organisms. The active ingredient of the antidepressant Prozac™, fluoxetine (FLX), is detected in aquatic environments at concentrations as high as 0.54 $\mu g \cdot L^{-1}$. Fluoxetine is a selective serotonin reuptake inhibitor and since serotoninergic systems are highly conserved, the presence of FLX in aquatic environments may result in unintended effects to aquatic vertebrates. In this study, we investigated the transgenerational effects of environmental FLX concentrations on the stress axis of zebrafish (ZF), *Danio rerio*. Embryos/larvae were exposed to either control or two concentrations of FLX: 0.54 $\mu g \cdot L^{-1}$ and 54 $\mu g \cdot L^{-1}$ from 3 hours post-fertilization (hpf) to 6 days post-fertilization (dpf). The stress response was assessed by examining the physiological and behavioural effects following standardized net and novel tank stressors, respectively, at adulthood (6 months). Fluoxetine exposure decreased basal and stress-induced whole-body cortisol levels at 6 months in a concentration-dependent manner ($p<0.05$). Additionally, these FLX-exposed fish did not respond to an i.p. injection of 0.0625 IU ACTH ($p<0.05$), suggesting that the interrenal cells may be a target of disruption by FLX. The FLX-treated fish displayed more anxiety-like behaviours (novel tank response) compared to controls ($p<0.05$). A total of 38.2% and 4.4% of the offspring of the 0.54 $\mu g \cdot L^{-1}$ and 54 $\mu g \cdot L^{-1}$ FLX-treated parents, respectively, exhibited a white-body phenotype, which was not observed in the controls ($p<0.05$). These results demonstrate that early life exposure to FLX not only disrupts the ZF stress system but it also interferes with the pigmentation of the offspring of exposed parents. The blunted stress response and the lack of pigmentation observed in the offspring of the FLX-treated fish persisted across generations. Supported by the FRQNT scholarship (MVC) and grants from the National Sciences and Engineering Research Council (TWM, VLT) and University Research Chair Program (VLT).

Effects of the novel brominated flame retardant 1,2,5,6-tetrabromocyclooctane (TBCO) on reproductive capacity of 2 generations of Japanese medaka (PL)

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The novel brominated flame retardant (NBFR) 1,2,5,6-tetrabromocyclooctane (TBCO) is a potential alternative to the major use brominated flame retardant, hexabromocyclododecane (HBCD). In a 21-day reproduction assay, fecundity of Japanese medaka (*Oryzias latipes*, F0 generation) fed a diet containing 58 μg TBCO·g^{-1} food (F0-TBCO) was 30% less than fecundity of controls (F0-Control). Concentration of TBCO in eggs from F0 TBCO fish was 14.3 μg·g^{-1}, demonstrating maternal transfer of this chemical. To evaluate effects of maternally deposited TBCO on reproductive capacity of F1 adults, embryos collected from F0-Control and F0-TBCO adults were reared in freshwater and given a control diet until sexual maturity. Adults raised from embryos from F0-Control fish and F0-TBCO fish were termed F1-Control and F1-TBCO, respectively. In a 21-day fecundity assay the F1-Control adults were fed either a control diet or a diet containing 58 μg TBCO·g^{-1} food, and these fish were denoted F1-Control/Control and F1-Control/TBCO, respectively. Also, F1-TBCO adults were fed either a control diet or a diet consisting 58 μg TBCO·g^{-1} food, and these fish were denoted F1-TBCO/Control and F1-TBCO/TBCO, respectively. Fecundity of F1-Control/TBCO was 16% less than fecundity of F1-Control/Control, which is consistent with results of the reproductive assay with F0 fish. However, fecundity of F1-TBCO/Control fish was 20% less compared to F1-Control/Control. This suggests that exposure to TBCO that was maternally deposited into embryos might have stimulated molecular programming that affected reproductive capacity at sexual maturity. Interestingly, fecundity of F1-TBCO/TBCO fish was equal to F1-Control/Control fish and 25% greater than F1-TBCO/Control fish, which suggests that re-exposure to TBCO during reproduction might have stimulated reproductive capacity. The molecular basis of altered reproductive capacity of adults exposed to TBCO via maternal deposition and upon re-exposure is being investigated.

Maternal transfer of bisphenol A disrupts the liver proteome of rainbow trout in multiple generations (PL)

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Bisphenol A (BPA), an organic contaminant, is widely distributed in the environment. It accumulates in fish tissues, and exposure to this contaminant affects growth and development. BPA is an established endocrine disruptor, and in fish is weakly estrogenic. There is evidence that BPA may cause epigenetic modifications; however, the multigenerational consequence of BPA exposure is not known in fish. We tested the hypothesis that maternal transfer of BPA leads to liver dysfunction and this effect is carried over in multiple generations. We utilized a proteomics approach to identify pathways that may indicate liver dysfunction. To mimic maternal transfer of this contaminant, oocytes were enriched with environmentally-realistic concentration of BPA (0, 4 or 40 ng per egg), after which they were fertilized with clean milt and reared in clean water. BPA persisted in the embryos, but was completely cleared from all groups at hatch (42 days post-fertilization (dpf)). Liver tissue was sampled at 140 and 365 dpf, both from the F1 and F2 generation trout. Liver proteome was analyzed using the isobaric tags for relative and absolute quantitation (iTRAQ) method. BPA disrupted the liver proteome of trout in both generations. The impact of BPA was more pronounced in the F2 generation; at 140 and 365 dpf there were over 20 proteins, including glutathione S-transferases and cytochrome P450, that were differentially expressed in response to BPA. Overall, these results reveal a multigenerational impact on liver proteome associated with maternally transferred BPA in rainbow trout (*Oncorhynchus mykiss*). We propose epigenetic modifications as a possible mechanism for this effect of BPA in multiple generations.

Airborne exposure of polycyclic aromatic hydrocarbons (PAHs) in Hamilton Harbour, Ontario, affects global DNA methylation in wild double-crested cormorants (*Phalacrocorax auritus*) (PL)

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Polycyclic aromatic hydrocarbons (PAHs) are known and probable carcinogens that are emitted into the environment through the incomplete combustion of fossil fuels and steel production. Studies have shown that exposure to PAHs in the environment can induce both epigenetic toxicity and genotoxicity, but few studies have related PAH exposure to changes in the putative molecular pathways in free ranging wildlife. Previous work has suggested that double-crested cormorants (*Phalacrocorax auritus*) had a higher incidence of genetic mutations at sites in Hamilton Harbour, Ontario, Canada, where higher PAH concentrations in the air and sediment occur. The aim of this project was to determine if PAHs altered the level of global DNA methylation (5-methylcytosine) and global DNA hydroxymethylation (5-hydroxymethylcytosine). An additional research objective was to assess whether PAHs alter the mRNA concentrations of seven genes involved in the TP53 (tumour suppressing gene) pathway. Liver and lung tissue samples were collected from *P. auritus* at sites in Hamilton Harbour and Lake Erie with differing levels of PAH exposure. Global DNA methylation was decreased by 50% in the liver of birds from the most PAH-contaminated site compared to the control, whereas preliminary results show less of a decrease in the lungs. A decrease in global DNA methylation with exposure to PAHs has also been shown in humans and fish. As an intermediate in DNA demethylation pathways, we would also expect global DNA hydroxymethylation to decrease with increased exposure to PAHs. Results from real-time RT-PCR show that expression of genes involved in the apoptosis pathway (liver tissue), DNA repair pathway (both tissues), and the regulation of p53 (lung tissue) were significantly different in the most PAH-contaminated site. Altogether,

data suggest that PAHs alter global DNA methylation in cormorants and activate the TP53 pathway, which allow birds to cope with increased exposure to mutagens.

Genetic polymorphism and epigenetic evidence from animals and humans to advance the risk assessment of mercury (PL)

Nil Basu[1]
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The risk assessment of mercury is complicated due to innate inter-individual variation in exposure, biomarker values, and adverse outcomes. Recent findings by us and others suggest that epigenetic mechanisms and gene-environment interactions may help explain some of this variation. For epigenetics, studies on laboratory models (mink, chicken, perch) and wild animals (polar bears) reveal mercury-associated global DNA hypomethylation and impaired DNMT activity in some, but not all, organisms. Candidate gene studies in a cohort of dental professionals reveals that methylation of SEPP1 is associated with significant changes in hair mercury levels in males only. In terms of gene-environment interaction studies, work by us and others show that some genes that influence the absorption, distribution, metabolism and elimination (ADME) of mercury have polymorphic variants that can affect biomarker levels. For example, epidemiological research by our group has shown that polymorphisms in MT1A, MT1M, GSTP1, and SEPP1 can be associated with altered levels of urine and hair mercury. For both epigenetics and gene-environment interactions, high-throughput approaches are commercially available which we are now exploring in a range of studies. This presentation will provide a rationale by which ecogenetic and molecular understanding, in both human and animal models, can be used to co-inform one another and also to improve our ability to assess the risks (both exposure and adverse outcomes) of mercury and help identify vulnerable groups or species.

Role of DNA methylation in the regulation of CYP1A induction by polycyclic aromatic hydrocarbons (PO)

Jonas Brandenburg[1] **and Jessica Head**[1]
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Gene expression is regulated in many different ways, including by epigenetic mechanisms such as DNA methylation. The objective of this study was to evaluate the capacity of polycyclic aromatic hydrocarbons (PAHs) to change the DNA methylation patterns of genes commonly induced by PAHs and other contaminants. We are also interested in whether an epigenetic mode of action of PAHs can persistently change the capacity of an organism to respond to contaminants throughout its lives. In this study, we focused on the CYP1A family of mixed function oxidases, using the chicken embryo as a model. Fertilized eggs were injected with PAHs prior to incubation and liver samples were taken at embryonic Day 10 (ED10) and 2 days post-hatch. CYP1A4 and CYP1A5

mRNA expression were assessed by qPCR. As expected, both genes showed a transient induction (27- fold and 16-fold) at high doses (greater than LD_{50}), but also at doses that do not cause embryomortality (4 -fold and 2-fold). No changes in global DNA-methylation were detected at either time point using LUminometric Methylation Assay (LUMA) analysis. In addition to global DNA methylation, the DNA methylation status of the shared CYP1A4 and CYP1A4 promoter was assessed by Bisulfite - Pyrosequencing. Further investigations will address the question of whether *in ovo* exposure to PAHs modifies an individual's capacity to induce CYP1A gene expression upon re-exposure to PAHs.

Municipal Wastewater

Characterization of the endocrine potencies of municipal effluents across Canada using *in vitro* bioassays (PL)

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Recent years have witnessed increasing concerns regarding the presence of contaminants in the environment that have the potential to adversely affect the endocrine system of humans and wildlife. One of the major sources for these endocrine disrupting chemicals (EDCs) in surface waters are municipal wastewater effluents (MWWEs), as conventional wastewater treatment technologies are frequently inefficient at removing these compounds from raw sewage. However, our understanding of the contribution of effluents from wastewater treatment plants (WWTPs) to environmental endocrine disruption in Canadian surface waters is incomplete. Therefore, the aim of this project was to investigate the effectiveness of six WWTPs across Canada to remove EDCs from MWWEs. Specifically, samples of influents and effluents were collected during spring, summer, and fall in 2014 and winter in 2015 to evaluate the influence of climatic conditions, population size, and treatment level on EDC removal efficiency. Endocrine potentials of wastewater were analyzed using three *in vitro* bioassays: MVLN ((anti-)estrogenicity), MDAkb2 ((anti-)androgenicity), and the H295R Steroidogenesis Assay (steroidogenesis disruption). Preliminary results indicated that effluent samples collected in summer 2014 from Regina (Saskatchewan), Saskatoon (Saskatchewan) and Guelph (Ontario) WWTPs had a significant increase in estrogenicity relative to the solvent controls at the highest concentration (10X). All influent samples collected in spring and summer 2014 had a significant increase in androgenicity relative to the solvent controls (4- to 5-fold), while effluent samples were less potent and showed relatively great variability in response, with the exception of samples collected from Montreal (Quebec) WWTP in summer 2014, where androgenic activity was detected. Removal efficiency of androgenic activity was greatest for Quebec (Quebec) WWTP (89%) and least for the Montreal (Quebec) WWTP (52%) in spring 2014, suggesting different efficiencies of treatment processes. This project will provide insights into the most effective approach for monitoring MWWEs, and will inform development of advanced wastewater treatment technologies for improved removal of EDCs.

Thyroid hormones and thyroid hormone disruptors and their effects on chemosensation in North American bullfrog (*Lithobates catesbeianus*) tadpoles (PL)

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Endocrine disrupting chemicals (EDCs) in wastewater effluents (WE) are a growing concern in water quality today. Endocrine disrupting compounds enter wastewater through many anthropogenic sources, including personal care products, pharmaceuticals, antimicrobials, and detergents. These compounds are not fully removed by wastewater treatment processes, and therefore are present at low concentrations in wastewater effluents. Endocrine disrupting compounds can act as agonists or antagonists to the thyroid hormone (TH), resulting in the thyroid hormone system being disrupted. Thyroid hormone is important to vertebrates for normal growth and development of a variety of tissues and organs, including the brain. This study investigates the effects of EDCs on chemosensory acuity in North American bullfrog (*Lithobates catesbeianus*) tadpoles. Premetamorphic tadpoles were exposed to environmentally relevant concentrations of THs thyroxine (T4), triiodothyronine (T3), and 17β-estradiol (E2), as well as known EDCs ibuprofen (IBF) and triclosan (TCS) in the presence and absence of T3 and T4. Chemosensory acuity is measured using behavioural and electro-olfactography (EOG) endpoints. Choice trough maze assays were used to indicate the way an odour is detected and perceived by the tadpole, which is demonstrated by its ability to respond appropriately to chemosensory food cues. Electro-olfactography is a neurophysiological technique that measures the strength of the response from olfactory tissue after an odour is detected. The same chemosensory food cue was used to stimulate behaviour and EOG responses, providing a link between both chemosensory endpoints after TH and EDC exposures. Amphibians are excellent sentinels for studying the effects of TH disruption by EDCs because metamorphosis relies on proper TH signalling. The effects of TH disruption on chemosensory acuity are an indication of neurophysiological responses, which can have important ecological implications. Changes in chemosensory acuity could impact an organism's ability to locate food, avoid predators, and find mates. Studying changes in chemosensory acuity will provide a greater understanding of the ecological significance of EDCs present in wastewater and the implications their presence may have on amphibian populations in receiving waters.

Reproductive and histopathological effects of municipal wastewater effluent exposure in male and female fathead minnows (PL)

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Municipal wastewater effluents (MWWEs) contain anthropogenic substances that can exhibit endocrine disrupting activity. In the Prairie provinces, increasing urban populations and industrial activities has meant greater water demand and therefore greater MWWE released into the aquatic environment. The objectives of this study were to (1) determine the biological effects of MWWEs on fathead minnow (*Pimephales promelas*) using the U.S. Environmental Protection Agency short-term fish reproductive bioassay and (2) compare responses to effluents collected from both the Regina and Saskatoon wastewater treatment systems. Fish were exposed for 21 days to 0%, 10% and 50% effluent. The endocrine disrupting effects were assessed using various reproductive measurements (number of eggs, fertilization success), as well as morphological, histopathological, and molecular/biochemical indicators in both male and female fish. Exposure of fathead minnows to Regina MWWE caused a significant decrease in fecundity at all concentrations compared to non-exposed minnows. Fish exposed to Regina MWWE caused a trend of lowered gonadosomatic index (GSI), although this effect was non-significant in male fathead minnows. Fecundity was not affected by exposure to Saskatoon MWWE; however, male minnows exposed to Saskatoon MWWE exhibited decreased liver somatic index (LSI). Fertilization rate remained consistent regardless of treatment. Histological examination revealed an increase in proportion of spermatogonia in male fathead minnow testes in 50% effluent concentrations compared to control minnows in both Regina and Saskatoon exposures. Increased testicular degeneration and interstitial cell hypertrophy was observed in male fish exposed to Regina and Saskatoon high dose effluent treatment compared to controls. This study confirmed the reproductive impacts of MWWE discharged into Saskatchewan aquatic systems and will provide critical information regarding the particular toxicological risks associated with ineffective removal of endocrine active compounds from MWWEs. (Funded by CWN.)

Assessing potential endocrine disrupting effects of municipal effluents on fathead minnow (*Pimephales promelas*) populations in Southern Saskatchewan (PL)

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The province of Saskatchewan is experiencing a dramatic increase in population growth, resulting in a greater release of municipal wastewater effluents (MWWEs) into

local water bodies. There is concern regarding the impact of contaminants in this effluent, particularly endocrine disrupting compounds (EDCs), to resident wildlife as conventional wastewater treatment technologies are often incomplete or inefficient at removing such compounds. Waterbodies in the southern Canadian Prairies may be at an elevated risk of exposure to EDCs due to the uniqueness of prairie surface water systems. During low flow periods, Wascana Creek, a small stream in southern Saskatchewan, can consist of over 90% treated effluent originating from the City of Regina's outdated lagoon-based treatment facility. The aim of this study was to assess the potential endocrine disrupting effects of MWWEs on populations of fathead minnows (*Pimephales promelas*) that inhabit Wascana Creek, Saskatchewan. Fathead minnows were collected during the spawning season (August 2014) to assess overall health (condition factor, somatic indices), reproduction endpoints (secondary sexual characteristics, concentrations of sex steroids in blood plasma, gonadal morphology and histology, and gene expression), and sex ratios. Fish collected downstream of the effluent fallout had lower gonadosomatic indices and significantly greater hepatosomatic indices compared to fish from upstream populations. Additionally, there was significant disruption of regulation of key genes along the hypothalamus-pituitary-gonad-liver axis that are associated with reproductive processes. Finally, male fathead minnows collected downstream displayed lower scores of secondary sexual characteristics. Preliminary data supports the hypothesis that MWWEs might be adversely impacting populations of fathead minnows inhabiting receiving waters around Regina, thus warranting further research.

Annual variability in biological measures in response to chemicals of emerging concern (PL)

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It is well established that exposure of aquatic organisms to chemicals of emerging concern (CEC) contained in municipal wastewater effluent (MWWE) can have deleterious effects on the health of fish populations. While this has been well demonstrated in the laboratory, it remains difficult to associate CEC in MWWE with impacts on the health of wild fish populations. This difficulty can be attributed to municipal wastewater treatment plant outfalls being located in urban areas, which present additional stressors for freshwater systems. Thus, it can be difficult to determine whether impacts observed in aquatic organisms are due to MWWE, or another stressor. The purpose of this study was to evaluate biological indicators of CEC exposure by observing their annual variability in a wild population of fish exposed to MWWE. The system used to conduct this study was the central reach of the Grand River in Kitchener, Ontario, where two municipal wastewater treatment plants are located. Rainbow darter (*Etheostoma caeruleum*) were collected from sites across an urban gradient in the spring and fall between 2007 and 2013, with four years of data for each

season. Analysis of selected pharmaceuticals, liver gene expression, gonadal sex steroid production, gonad histology, somatic indices, and sex ratio were compared across years and seasons. The data demonstrate that some measures are more variable annually than others. There was a clear indication of CEC exposure downstream of the two MWWE outfalls in gonad histology metrics in fall, but not spring collections. This study provides support for the use of gonad histology in widespread surveys of fish exposure to CEC in MWWE, with the caveat that life history of a fish species may alter the interpretation of the source of exposure.

Reduction in intersex incidence and severity in a wild fish population after infrastructure upgrades at a municipal wastewater treatment plant (PL)

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Impacts on fish in waters receiving treated municipal wastewater treatment plant (WWTP) effluents is a concern globally, with endocrine disruption being one of the most prominent effects observed. The objective of this study is to assess whether major treatment upgrades at a WWTP will alleviate endocrine disruption and other impacts previously observed in wild fish. The WWTP located in Kitchener, Ontario, discharges effluent into a heavily urbanized reach of the Grand River. Multiple years of data have been collected for a sentinel fish species, the rainbow darter (*Etheostoma caeruleum*), associated with the Kitchener WWTP outfall since 2007. Endocrine disruption (intersex and decreased androgen production) in the male rainbow darter and altered ability to store and utilize energy, as indicated by somatic indices (increased condition and relative liver size, and reduced relative gonad size), were observed prior to the upgrades. Between 2012 and 2013, major upgrades took place in the plant to allow effluent nitrification, UV disinfection, and extended solids retention. Effluent quality improved with declines in contaminants such as nutrients (e.g., ammonia) and select pharmaceuticals, which are also reflected in the river water downstream the WWTP. The effluent estrogenicity was measured as high as 17 ng·L^{-1} total 17β-estradiol equivalents (Yeast Estrogen Screen) prior to treatment changes but has dropped dramatically with implementation of the upgrades. This improved effluent quality was also reflected in the food web with $\delta^{15}N$ values measured in fish tissue which are now similar to fish from upstream sites. Intersex condition in males downstream the Kitchener WWTP was still prevalent in the fall of 2013 and 2014; however, percent incidence and severity dropped by as much as 75%, suggesting that the rainbow darter populations are responding to process upgrades. This study is a unique opportunity to assess the impacts of infrastructure upgrades of WWTPs on effluent quality and biological responses in wild fish.

Assessing the impact of wastewater effluents in the Grand River, Ontario (PL)

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Municipal wastewater represents one of the largest sources of effluent to aquatic ecosystems across Canada. These effluents contain a diversity of contaminants, including those that can alter endocrine function and reduce reproductive performance of fish. Studies have been conducted in the Grand River, Ontario, on rainbow darter (*Etheostoma caeruleum*) since 2007 to assess impacts on multiple levels of biological organization associated with several municipal wastewater treatment plant (WWTP) effluents. The treatment plants represent a diversity of treatment systems, sizes, and effluent dilution. Since these initial studies, some of the treatment plants have undergone a number of significant process changes, especially in the Kitchener WWTP that now nitrifies the effluent. This change is reflected in the chemical profiles of nutrients and contaminants such as pharmaceuticals, as well as effluent toxicity. Total estrogenicity (based on receptor assays) of the Kitchener effluent has declined sharply since 2011-12 and this is reflected in decreasing intersex incidence and severity as well as changes in gene expression (i.e., transcriptomes) and gonad *in vitro* steroid production relative to the reference sites. Effects at higher levels of biological organization, such as somatic indices, are much more variable and difficult to separate from natural variability, although there are indications that, prior to upgrades, there were disruptions in energy storage and utilization (e.g., condition, liver and gonad somatic indices). Fish community assemblages are also highly variable but our data suggest that observed shifts in species abundances are associated with poor quality effluents. These integrated studies have implications for environmental regulation and design of future biomonitoring programs.

Endocrine disruption of emerging compounds in municipal wastewaters: Impacts in the St-Lawrence River (PL)

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Urban wastewater effluents were collected from two large cities releasing into the St-Lawrence River. In the laboratory, fathead minnows were exposed over sixteen weeks to a series of dilutions of the effluents and a range of biomarkers of endocrine disruption, reproductive capacities, immunotoxicity and genotoxicity were examined. Fish, water, and sediments were also collected in the field, in areas suspected to be

impacted within the St-Lawrence River. The chemical characterization of water and sediments included a series of thirty endocrine disruptors (hormones, antidepressant drugs, plastic by-products, illicit drugs among others) analyzed within the dissolved phase and also within the suspended particulates—thus allowing an estimation of solid-liquid fractionation. Fifteen of the target compounds were detected in the effluents, in concentrations that varied over the sixteen weeks and were systematically different in the two target cities' effluents—presumably because of different ratios of rainwater infiltration and aqueduct losses. Preliminary results from enzyme analyses and qRT-PCR analysis seem to indicate that adult fathead minnows do not display endocrine disruption at dilutions up to 20% effluent in the laboratory. A modulation of immune capacity was observed in juveniles after effluent exposure, whereas adults did not show immunotoxic response. The two effluents were found to be genotoxic. Wild fish, however, do display significantly depressed $\delta^{15}N$, indicating significant exposure to effluent derived particles in their diet. This result was correlated with immunotoxic response in round goby and yellow perch sampled in the effluent dispersion plume.

Assessment of environmental risks related to contaminants in municipal wastewaters: A toolbox approach (PL)

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Diverse chemicals in municipal wastewater treatment plant (MWTP) effluents have the potential to disrupt the normal function of endocrine systems and impair reproduction and development in fish and other organisms. Approaches are needed to understand whether these chemicals are causing biological effects downstream of MWTPs, and to prioritize sites for further work. This collaborative program was designed to assess a toolbox consisting of *in vivo* and *in vitro* assays as a sensitive screening approach for identifying the risks and magnitude of impacts in fish living in the immediate vicinity of these discharges. The overall goal was to identify a suite of measures that provide clear indications that biological effects are occurring as a result of the presence of effluent discharges, and a practical basis for distinguishing the relative level of concern among sites. A suite of endpoints were examined in wild fishes collected at reference and MWTP-impacted sites in the Grand River, Ontario, the Saskatchewan River and Wascana Creek in Saskatchewan, and the St. Lawrence River in Quebec, with a particular focus on those that can be used to understand endocrine-related impacts (i.e., hormones, vitellogenin, intersex), tissue and whole organism responses (i.e., condition, liver and gonad size) and exposure (i.e., stable isotopes, priority chemicals) of the fish to the contaminants. In addition, toxic potential of effluents was assessed using *in vitro* and *in vivo* bioassays with the aim to serve as efficient and cost-effective preliminary screening tools for the identification and prioritization of sites for more comprehensive *in situ* investigations. Results are being compared across sites and regions to understand those measures that distinguish sites of higher and lower concern based on the relative impacts observed at each downstream site compared to a reference site. This program will provide a suite of measures and associated recommendations that can be used to understand and compare biological effects of MWTP effluents among sites and to identify locations where interventions should be considered.

Using weight of evidence to evaluate potential effects of trace organics on aquatic life (PL)

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Thousands of trace organic compounds (TOrCs) are regularly released into the aquatic environment, and little is known of the effects of this complex mixture, although targeted studies suggest exposures could have important impacts on aquatic communities. Because of uncertainty concerning potential risks, management actions might focus on the wrong sites and/or compounds, and might not yield expected improvements. Establishing a causal linkage between wastewater treatment plant (WWTP)-related TOrC exposures and biological impairments is challenging due to the unsettled state of science with respect to which TOrCs are endocrine disruptors; what fraction of the TOrCs may be bioavailable; how TOrCs interact with other stressors; and whether the presence of TOrCs is a reliable indicator of aquatic life impairment. All these confounding considerations suggest there may be very different exposure and effects in an actual receiving waterbody than are indicated by screening models and laboratory studies. The approach we are piloting for the Water Environment Research Foundation screens WWTP discharges and assigns each to a risk- and action-based Tier of Concern, based on documented impairments and site stressors, level of wastewater treatment, dilution by receiving water, concentrations of TOrC indicators, and site-scale, screening-level evaluations of potential risk to aquatic life. For sites where existing data suggest moderate or higher levels of concern, field work is recommended to support a weight of evidence evaluation, including discharge and receiving water sampling, fish and macroinvertebrate bioassays, toxicity identification evaluations, and habitat assessments. The goal of the weight of evidence assessment is to allow WWTPs to better assess risk and potential causation to inform monitoring, planning, and wastewater treatment investments. The presentation will report on pilot field studies conducted at three sites in summer 2014.

Occurrence of wastewater contaminants at Norway House Cree Nation, Manitoba, Canada (PO)

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The presence of wastewater contaminants from Norway House Cree Nation's wastewater facility was characterized so that the local First Nations community could compare their exposures and releases to those of other communities. Water was sampled at four sites throughout the lagoon and treatment facility, downstream of the discharge into Little Playgreen Lake, and at an upstream control site that also serves the community's drinking water source. Nutrients (nitrogen and phosphorus) and basic water quality parameters (e.g., pH, temperature, conductivity and dissolved oxygen) were measured at all sites. While nutrients were effectively removed from tertiary lagoon to final effluent, no improvement in water quality or reduction in nutrients was observed within the primary, secondary, and tertiary lagoons. Pharmaceuticals and pesticides were measured by passive sampling using Polar Organic Chemical Integrative Samplers (POCIS). Antibiotic-resistant genes (ARGs) were monitored for via grab sampling. Eight of 42 compounds were detected at the four sites throughout the treatment facility: atenolol, atrazine, carbamazepine, sulfamethoxazole, sulfapyridine, gemfibrozil, metoprolol and trimethoprim. Pharmaceutical concentrations at upstream and downstream sites were below detection limits, indicating no exposure via drinking and recreational waters. Reduction of most compounds from lagoon to final effluent was limited. Atrazine was found at all sampling sites, with the greatest concentrations found at upstream and downstream sites (ranging from 16-18 $ng{\cdot}L^{-1}$). Antibiotic resistance genes (ARGs) from the water samples showed the greatest abundances of tetR (sum of tetracycline resistance genes) in the tertiary lagoon, representing a ten-fold increase from the upstream and downstream sites. Further gene abundances and bacterial loads will be quantified to assess ARG spatial behaviour within the treatment plant. With these preliminary results, more sampling points will be collected to further aid in assessment of temporal and spatial trends, and another polar passive sampler will be deployed in parallel to the existing POCIS for field validation purposes.

Transcriptome profiling in the liver of wild rainbow darter (*Etheostoma caeruleum*) in the central Grand River, Ontario, to assess impact and recovery of resident populations (PO)

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The central Grand River in southern Ontario receives diverse anthropogenic inputs including discharges from both the Waterloo wastewater treatment plant

(WWTP) and Kitchener WWTP. The Kitchener WWTP has undergone significant system upgrades which have resulted in improved effluent quality. There is considerable evidence that contaminants of emerging concern can elicit sublethal effects that can be detected at the molecular level in fish residing in this urbanized area of the Grand River. To further our understanding of site-specific contaminant effects, male rainbow darter (*Etheostoma caeruleum*) (RBD) were collected from nine sites at varying distances from the WWTPs and a microarray used to assess hepatic gene expression using a spatial gradient approach. The first objective was to determine if RBD gene expression profiles at the Kitchener and Waterloo WWTPs would be different from individual profiles at the furthest upstream (reference) site. The second objective was to compare differently expressed genes in individuals between Waterloo and Kitchener WWTPs, and to determine if Kitchener WWTP upgrades affect the hepatic transcriptome. Lastly, a major objective was to determine if the impacts in the urbanized area diminish with distance from sources of contamination. Gene expression profiles suggested that there are different gene expression patterns in fish exposed to Waterloo WWTP discharges compared to the reference site, and this difference is more subtle in fish exposed to Kitchener WWTP effluent. There were 3340 differently expressed probes (DEPs) in individuals from Waterloo WWTP compared to the reference site; other sites had a lower number (i.e., less than 2036) of DEPs compared to the reference site. This suggests that, following the upgrades, there are fewer differences in the transcriptome compared to those from the reference site. Subnetwork enrichment analysis (SNEA) showed that male RBD exposed to Waterloo WWTP had altered genes associated with lipid metabolism, stress responses, immunity and reproduction. Based on SNEA, there were some indications of similarities in responses between recovery sites and the control site and this is being explored in more detail.

Non-conventional Oil and Gas

In vitro and *in vivo* oxidative stress responses of oil sands process-affected water (PL)

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There is concern about adverse effects of oil sands process-affected water (OSPW) on aquatic organisms. However, mechanisms by which OSPW causes adverse effects are not fully understood. There is evidence that dissolved organic chemicals in OSPW might cause toxicity by oxidative stress, but definitive studies linking exposure to OSPW with adverse effects caused by oxidative stress have not been performed. In addition, chemicals that cause oxidative stress have not been identified. In this study, dissolved organic chemicals in OSPW were extracted by use of hydrophilic-lipophilic balance (HLB) cartridges and fractionated sequentially by use of hexane (Hex), 20% dichloromethane (DCM) in Hex, 50% DCM in Hex, 100% DCM, and 100% methanol (MeOH). *In vitro* and *in vivo* assays were used to screen fractions of OSPW for potential to cause oxidative stress, and high resolution Orbitrap mass spectrometry was used to identify chemicals that might cause oxidative stress. Activation of nuclear receptor erythroid 2-related factor 2 (Nrf2), a key transcriptional regulator of the response to oxidative stress, was 2.9 ± 0.1-fold greater in cells exposed to a total extract (TE) of OSPW. Among the five fractions, activation of Nrf2 was 2.7 ± 0.1-, 1.2 ± 0.1-, and 1.7 ± 0.1-fold greater in cells exposed to the 20% DCM fraction 100% Hex, and 100% MeOH fractions, respectively. The Nrf2 was not activated in cells exposed to either 50% DCM or 100% DCM fractions. Activation of Nrf2 by TE or the 20% DCM fraction was reduced by 41.4% and 38.5%, respectively, when cells were co-exposed to 5 mM of reduced glutathione (GSH), a well-known anti-oxidant. Expression of genes that are important for the response to oxidative stress were quantified in Japanese medaka (*Oryzias latipes*) larvae exposed to fractions. Expression of superoxide dismutase (SOD), catalase (CAT), and glutathione-S-transferase (GSTP and GSTA) were significantly greater in medaka larvae exposed to the 20% DCM fraction, which is consistent with *in vitro* activation of Nrf2. Concentrations of lipid hydroperoxides (LPO) was 1.7 ± 0.3-fold greater in medaka larvae exposed to the 20% DCM fraction. Taken together, these results suggest that chemicals in the 20% DCM fraction might have caused oxidative stress. Analysis of this fraction by use of high-resolution Q-Exactive mass spectrometry and a non-targeted data mining strategy identified approximately 200 compounds with exact m/z values and retention time. Studies are currently in progress to identify the biochemical source of oxidative stress by quantification of reactive oxygen species. Overall, results from the current study provide additional evidence that OSPW might cause toxicity by oxidative stress.

Long-term effects in fathead minnow exposed to oil sands sediments early in life (PL)

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One of the theories that have been proposed in Canada's oil sands area is that early exposure of fish to oil sands related compounds in rivers may cause effects later in life, even after the fish grow and move to larger rivers far from oil sands. The aim of this study was to characterize potential effects of natural oil sands sediments on the fathead minnow in a lifecycle test that assesses growth, maturation, and reproduction. Fish were exposed for 22 days, from egg fertilization to 16 days post-hatch (dph) to 1 or 3 $g \cdot L^{-1}$ of river sediment from 3 different sites: a control site (alkyl polycyclic aromatic hydrocarbons (PAHs) Σ 3 $ng \cdot g^{-1}$ wet weight sediment), one site influenced by the natural oil sands (alkyl PAHs Σ 6200 $ng \cdot g^{-1}$), and one site with natural oil sands that is near mining development (alkyl PAHs Σ 4500 $ng \cdot g^{-1}$). After this embryo-larval exposure period, fish were transferred to clean water flow-through tanks and raised to adulthood. Deformities and reduced weight were observed during the exposure period, but growth recovered over time in clean water. Reproduction was decreased (significantly fewer eggs laid) in fish exposed to 3 $g \cdot L^{-1}$ sediment from the river near mining development. However, although there were effects on the early-exposed fish, there were no effects in their offspring as the F1 showed good hatching, survival, and very low incidences of deformities.

Assessing the impact of source and age of oil sands process-affected water on the toxicity of extracted naphthenic acids (PL)

Adrienne Bartlett[1], Richard Frank[1], Patricia Gillis[1], Lisa Brown[1], Mark Hewitt[1], Pamela Brunswick[1], Dayue Shang[1] and Joanne Parrott[1]
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Mixtures of acid-extractable organic compounds, including naphthenic acids (NAs), are concentrated in oil sands process-affected water (OSPW) through the extraction of bitumen from oil sands deposits in Alberta, Canada, and have been identified as being among the primary toxic components of OSPW. Previous studies have shown that the composition of complex NA mixtures impacts toxicity, and that processes including microbial degradation lead to a decrease in toxicity over time. Thus, the source and age of OSPW can influence effects on aquatic biota. The objective of this study was to assess the toxicity of NA mixtures extracted from varying sources of OSPW: "fresh" OSPW collected in different years from the same active tailings pond at Industry A (2009, 2011), fresh OSPW collected from an active tailings pond at Industry B, and "aged" OSPW collected from an inactive experimental tailings pond (OSPW more than 20 years old) at Industry A. Aquatic toxicity tests were conducted with *Vibrio fischeri*

(marine bacterium, Microtox® assay), *Lampsilis cardium* (freshwater mussel), and *Hyalella azteca* (freshwater amphipod). For *V. fischeri* and *H. azteca*, the toxicity of all four NA extracts was similar irrespective of source or age of OSPW. In contrast, for glochidia (larvae) of *L. cardium*, the toxicity of the NA extracts ranged 17-fold from most to least toxic: Industry A aged > Industry B fresh > Industry A 2009 fresh ~ Industry A 2011 fresh. The fact that the relative toxicity of these four NA extracts was inconsistent across the three test species suggests that extrapolation between species to estimate the toxicity of NA mixtures should be done cautiously, if at all. In research conducted previously, we demonstrated that NA commercial mixtures were more toxic to all three test species than NAs extracted from OSPW: up to 4-, 120-, and 30-fold more toxic for *V. fischeri*, *L. cardium*, and *H. azteca*, respectively. This indicates that NA extracts are a more relevant source for generating toxicity data for the derivation of NA water quality criteria.

Toxicity of aqueous vanadium to aquatic organisms relevant to the Athabasca Oil Sands region for use in development of water quality guidelines (PL)

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Bitumen from the Athabasca Oil Sands (AOS) region contains elevated concentrations of the metal vanadium (V). When bitumen is upgraded to synthetic crude oil, V is removed and enriched into the by-product coke. Currently, Syncrude Canada Ltd. generates up to 3 million tons of coke annually, which is stockpiled on-site. Past research has found that under wet conditions V leaches from coke at ecotoxicologically relevant aqueous concentrations. This suggests that coke leachates could expose aquatic ecosystems of northern Alberta to elevated concentrations of V, depending on future plans for storage and remediation of coke stockpiles. This research aimed to develop new V toxicity data to various freshwater algae, invertebrate, and fish species that are either commonly-used laboratory species, or species more regionally-representative of northern Alberta. Acute and chronic species sensitivity distributions (SSDs) were then developed using these new data along with existing, published data. Toxicity endpoints for V included lethality, growth, inhibition of reproduction, and emergence of insects. From the SSDs developed, acute and chronic concentrations hazardous to 5% of the species tested (HC5s) and associated 95% confidence intervals were 0.55 (0.45 - 0.66) and 0.08 (0.05 - 0.15) mg $V \cdot L^{-1}$, respectively. Currently, acute SSDs have shown regionally relevant species to be slightly more sensitive to V toxicity than standard test species. However, this difference in sensitivity has not been observed in the chronic SSD. Acute and chronic HC5s have been estimated at 1.20 and 0.25 mg $V \cdot L^{-1}$ and 0.11 and 0.04 mg $V \cdot L^{-1}$ for standard and regionally-relevant species, respectively. This research provides the data necessary for development of appropriate water quality guidelines for V for use in the AOS region, and for risk assessment of coke placement in reclamation landscapes.

The influence of water chemistry on the acute aqueous toxicity and speciation of vanadium (PL)

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Alberta's oil sands industry coke generation in 2014 was reported to be approx. 8.9 million tonnes, which contain up to 5% of vanadium (V) by weight. The use of coke as a sorbent to reduce concentrations of organic chemicals in oil sands process water (OSPW) has been recently investigated. Unfortunately, in the process V is released from the coke and can increase in "treated" OSPW to levels up to 7 $mg\cdot L^{-1}$ of V. Little work has been done to understand how common water quality variables can affect toxicity of V to aquatic organisms. Here the potential for adverse effects of V to aquatic organisms is described by developing site-specific relationships that model how different characteristics of surface waters representative of the Alberta oil sands region affect V toxicity to *Daphnia pulex*. Also, V speciation is modeled using Visual MINTEQ to better understand the effects of key constituents of OSPW on the toxicity of V. Results to date indicate that an increase in pH resulted in a threshold relationship where toxicity increased between pH 6 and 7, and then leveled off. In addition, when alkalinity (100 to 600 $mg\cdot L^{-1}$ $CaCO_3$) increased, the toxicity of V decreased following an inverse model. Also, when sulfate increased from 30 to 380 $mg\cdot L^{-1}$, the LC_{50} to *D. pulex* increased by 0.4 and 0.3 $mg\cdot L^{-1}$, following a quadratic model between 100 and 380 $mg\cdot L^{-1}$. However, changes in water hardness (70 to 140 $mg\cdot L^{-1}$ as $CaCO_3$) and chloride concentration (10 to 120 $mg\cdot L^{-1}$) did not have a modifying effect. Vanadium speciation was only modified by pH, and the presence and proportion of HVO4-2 and H2VO4- along the variables tested indicated that both species are responsible for V toxicity to *D. pulex*. Finally, the influence of a mixture of sodium and sulfate on V toxicity to *D. pulex*, simulating OSPW dilution into Athabasca River water, will be presented to simulate a more environmentally representative scenario.

Effects of diluted bitumen exposure on the cardiovascular system of juvenile sockeye salmon: From cells to performance (PL)

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Diluted bitumen (Dbit) is transported from the Athabasca Oil Sands across the continent by rail and pipeline. In British Columbia, existing and proposed pipelines cross prime freshwater habitats that are critical to the anadromous lifecycle of Pacific salmon species. Although cardiotoxicity has been reported in several teleost species exposed to crude oil fractions, there have been no studies on how Dbit exposure influences cardiac health, aerobic performance, and ultimately the migratory ability of any Pacific salmon species. Juvenile sockeye salmon (*Oncorhynchus nerka*) were exposed to the water-soluble fraction of Dbit (nominal total initial polycyclic aromatic hydrocarbon (PAH) concentrations of 0, 1, 10, and 100 $\mu g \cdot L^{-1}$) for 10 and 28 days, followed by an assessment of aerobic swimming performance using a ramp critical swimming speed (U_{CRIT}) test. At fatigue, plasma and hearts were collected for comparison to exposed but non-swum fish in each treatment group. As expected, liver ethoxyresorufin-O-deethylase activity was significantly elevated relative to control fish in all Dbit exposed fish at 10 and 28 days, confirming that PAHs were bioavailable and caused the induction of biotransformation enzymes. While there were no differences in U_{CRIT} values of fish at 10 days of exposure, sockeye exposed to the highest dose of Dbit had significantly lower U_{CRIT} values compared to all other fish following 28 days of exposure. Cardiotoxicity is specifically being addressed through histological comparisons of cardiac tissue integrity between the treatment groups. No evidence was found for an effect of either Dbit exposure or exercise on apoptosis in fish hearts (TUNEL assay). Proteomic analyses of plasma samples are also being evaluated to determine how Dbit exposure and exercise interact to influence whole animal physiological processes. Overall, our findings are important for understanding the impact that Dbit contamination in salmon habitats can have on salmon populations, particularly in terms of their migratory success. Supported by the National Contaminants Advisory Group of Fisheries and Oceans Canada.

Response of native amphibians to reclaimed wetlands in the Athabasca Oil Sands (PL)

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Oil sands mining companies in Canada are required by law to reclaim land mined or disturbed by their operations. Reclaimed wetlands are primarily constructed as marshes, while other reclaimed wetlands form opportunistically in reclaimed areas. Many of these wetlands contain oil sands process-affected materials that are highly saline and contain naphthenic acids, polycyclic aromatic hydrocarbons, and metals. These contaminants are known to affect wildlife; therefore, it is important to determine whether reclaimed wetlands containing such contaminants can support wildlife to the same extent as regional reference wetlands. Anurans were used as model species because their biphasic lifecycle makes them sensitive to contaminants. Thus, we examined the ability of reclaimed (constructed and formed) wetlands to support wood frog (*Lithobates sylvaticus*) and Boreal chorus frog (*Pseudacris maculata*) tadpoles using field and lab studies. We exposed tadpoles (Gosner stage 26-28) *in situ* to six reclaimed wetlands on an oil sands lease and two regional reference wetlands, and monitored for survival, growth, and development. In wetlands, tadpoles are naturally exposed to water and sediment; however, our *in situ* enclosures prohibited contact with sediment. In wetlands, sediment acts as a contaminant sink, thereby potentially serving as a contaminant source. We used mesh exclusions in a lab exposure to compare the toxicity of water and sediment collected from our eight field sites and determined if any observed effects are due to direct or indirect sediment exposure. Our research will determine whether these wetlands can support tadpoles throughout metamorphosis. Current results will be presented.

Exposure of fish in the Athabasca and Slave Rivers to PAHs potentially derived from petrogenic activities (PL)

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Polycyclic aromatic hydrocarbons (PAHs) are released to the environment from oil sands operations and from natural sources in Alberta, Canada. Concentrations of 16 U.S. Environmental Protection Agency (USEPA) priority PAHs and their alkylated analogues were measured in tissues of fishes collected from three locations on the Athabasca River in Alberta and two downstream locations on the Slave River in the Northwest Territories, Canada. A total of 425 individual fish were collected and included 89 goldeye (*Hiodon alosoides*), 93 whitefish (*Coregonus clupeaformis*), 104 northern pike/jackfish (*Esox lucius*), 96 walleye (*Sander vitreus*), and 43 burbot/loche mariah/mariah (*Lota lota*). Fish were sampled during the summer and fall of 2011, and spring of 2012. Dorsal muscle of fishes from upstream reaches of the Athabasca River, close to oil sands extraction and upgrading activities, contained greater concentrations of individual PAHs than concentrations in muscle of fishes from the Slave River. Concentrations of the sum of USEPA indicator PAHs (ΣPAHs) in fishes collected in the vicinity of Fort McKay, closest to oil sands activities, varied among seasons with average concentrations ranging from 11 (burbot, summer) to 1.2x102 ng·g^{-1} wet mass (wm) (burbot, spring) with a mean of 48 ng·g^{-1} wm. Concentrations of ΣPAHs in fishes collected in the vicinity of Fort Resolution, the location most distant from oil sands activities, also varied among species and seasons, with average concentrations ranging from 4.3 (whitefish, summer) to 33 ng·g^{-1} wm (goldeye, summer) with a mean of 13 ng·g^{-1} wm. Significant differences in concentrations of ΣPAHs in muscle were observed within goldeye, jackfish, walleye and whitefish among sites. Risks posed by PAHs to humans were assessed using a benzo[a]pyrene equivalents approach (BaP$_{eq}$). The average lifetime risk of additional cancers (LCR) for humans who consumed fish were deemed to be within the "acceptable" range of risk (i.e., less than 10^{-}*6*). PAH profiles suggest different sources of PAHs on the Athabasca and Slave rivers.

Investigations of oil sands influences on the Athabasca River Delta: PAHs, metals, and benthos (PL)

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Oil sands activities in the Fort McMurray area have been the subject of much investigation in recent years, particularly on the release of polycyclic aromatic hydrocarbons (PAHs) and metals to the environment through atmospheric and surface routes, and the possible impacts including on the Athabasca River and delta. Here we examine sediment and benthic data collected as part of the Regional Aquatic Monitoring Program (RAMP) over 1999-2013 to assess various temporal trends in the three primary Athabasca River delta channels and the lowermost site (ATR-ER) monitored on the Athabasca River. Total PAH concentrations averaged 1,029 ± 671 $\mu g \cdot g^{-1}$ at ATR-ER and were slightly higher (1,359 ± 359 $ng \cdot g^{-1}$) in the delta as flow velocity declined and suspended particulates began to settle. PAH composition profiles were similar to exposed bitumen beds and to sediments upstream of Fort McMurray with petrogenic sources generally indicated. Interim sediment quality guidelines were generally not exceeded and then most commonly for 2-methyl naphthalene among the PAHs and arsenic among the metals. PAH and metal concentrations were highly variable, primarily due to variations in sediment grain size and organic carbon. However, a small but significant amount of PAH variation in the delta was associated with a trend of temporal increase. Benthic communities were dominated by species characteristic of moving sediments and turbid environments, particularly Chironomidae and Tubificidae; these species generally are detritivores, obtaining their organic carbon from debris in the moving bed and suspended sediment load. There was a general temporal trend of declining benthic abundance and richness, possibly related to a trend of increasing sand content; this in turn could have resulted in the reduced delivery of organic carbon to the benthic community. Bioassay studies determined that growth and survivorship of *Chironomus* and *Hyalella* were good, with no spatial and temporal trends evident. Overall, temporal changes in the lower Athabasca River and the delta with the growing oil sands industry appear to be most strongly related to changes in sediment characteristic, with benthic communities appearing healthy.

Investigating the impacts of atmospheric emissions from Athabasca Oil Sands on adjacent boreal lakes through beta diversity patterns of zooplankton communities (PL)

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The fast pace and unprecedented scale of development of the oil sands industry in western Canada has raised concerns of potential acidification and nutrient enrichment of adjacent lake systems. We evaluated the beta-diversity patterns of crustacean zooplankton communities to evaluate the potential influence of acidifying/eutrophying emissions from the Athabasca Oil-Sand Region (AOSR) on boreal lakes in northern Alberta. Ninety-seven lakes representing five geographic clusters located on and off the Precambrian Shield (representing gradients in atmospheric deposition and landscape sensitivity) were surveyed for zooplankton and water chemistry parameters in 2012 as a part of Clean Air Regulatory Agenda (CARA) lake acidity study conducted by Environment Canada. Water chemistry parameters including alkalinity, dissolved organic carbon, nitrate (NO_3^-), sulfate (SO_4^{2-}) and aluminum (Al) varied significantly among geographic clusters, likely due to differences in underlying geology, catchment characteristics, and atmospheric deposition. In general, variations in water chemistry parameters were high in off-Shield lake clusters relative to those on-Shield. As expected, the relative contributions of species-replacement and -richness components to total beta-diversity of zooplankton communities did not vary among the five lake clusters. However, total beta-diversity of the on-Shield lake cluster located fairly close to the AOSR was considerably lower than that of other lake clusters. Interestingly, *Leptodiaptomus minutus*, a species dominant in culturally acidified lakes, contributed mostly to beta-diversity in this lake cluster, with variation in its abundance associated with Al concentration. On the other hand, environmentally related variation in beta-diversity was largely similar among the other lake clusters. In these clusters, greater abundances of *Daphnia pulex* were associated with higher concentrations of NO_3^-, SO_4^{2-} and fishless conditions, whereas the abundances of *Bosmina longirostris* were negatively related to these conditions. Meanwhile, abundances of acid-sensitive *Skistodiaptomus oregonenensis* were positively related to alkalinity in these clusters. Our findings suggest that beta-diversity patterns of zooplankton communities across the study region are likely influenced by interactions between landscape sensitivity and atmospheric deposition.

Constraints on biodiversity and ecosystem functioning in the Athabasca oil sands: Using gamma irradiation to detoxify fine fluid tailings waste (PL)

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Extraction of bitumen from the Athabasca oil sands produces an enormous volume of slurry waste termed fluid fine tailings (FFT). Tailings are held in settling ponds where contaminated sand, silt, clay segregate prior to further dewatering to be used in reclamation efforts and where oil sands process water (OSPW) can be recycled for further use in the plants. The recycling of OSPW enriches the water with impurities such as naphthenic acids, salts and metals. One of the greatest restrictions imposed on oil sands companies is implementing an economically and environmentally viable way to manage the billions of cubic meters of tailings waste that continues to accumulate. Two major components concerning tailings are elevated salinity and concentrations of naphthenic acids, which are acutely and chronically toxic to aquatic and terrestrial biota. Our research involves assessing the efficacy of a novel method of detoxifying oil sands tailings using gamma irradiation (GI), breaking down the chemical bonds of more persistent forms of naphthenic acids. Current research is assessing GI as a field treatment by tracking the colonization of zooplankton, invertebrate and plant assemblages within 68-L microcosms constructed in September 2014 adjacent to a local wetland. Each microcosm was provisioned with 20 L of sediment and 26L of water from a single wetland and inoculated with 1.5 L of composite sediment and water created by mixing together equal quantities of material from each of the source wetlands and tailings ponds. The benthic and planktonic communities developing in microcosms containing GI treated and untreated sediment and water from 3 fresh wetlands, 3 saline wetlands, and 3 tailings ponds were sampled in spring 2015. All microcosms except those containing untreated tailings contained winged aquatic insects (dytiscid beetles and hemipterans) as well as larval dytiscid and chironomids. Submerged aquatic vegetation and algae were visible in microcosms with clear water. Development (percent cover) was somewhat slower in GI treated microcosms than in untreated microcosms. We will document the relationship between diversity (richness) of plant, zoo-benthos and zooplankton and net ecosystem productivity (NEP; inferred from diurnal changes in oxygen concentration) to elucidate the importance of species and functional group diversity on ecosystem functioning. This research provides an important field demonstration of the efficacy of gamma irradiation as a tailings treatment and contributes to the growing literature base on the effects of multiple stressors on communities as well as the relationship between community diversity and ecosystem services.

The complexity and challenges in assessment of toxicity of hydraulic fracturing flowback fluids (PL)

Yuhe He[1], Erik Folkert[1], Shannon Flynn[1], Daniel Alessi[1], Jonathan Martin[1] and Greg Goss[1]

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High-volume hydraulic fracturing (HF), as a revolutionary method of unconventional gas recovery, has drawn significant concerns about the potential environmental risk. Considerable attention has been given to contamination of shallow groundwater from deep reservoirs following fracturing. However, risk posed by HF flowback fluid (HFFF) has been less well studied. HFFFs, as a mixture of fracturing fluids and deep groundwater that returns to the surface following fracturing, are highly saline and have been demonstrated to contain heavy and/or radioactive metals, plus potentially thousands of organic constituents, which may be from the HF additives or naturally derived components. These complex fluids pose challenges for accurate identification of chemical profile and characterization of biological response. Risk of HFFF exposure to aquatic organisms stems from spills both on-site and during surface transport to disposal/treatment sites. The identification of the biological impact is challenging and the risk to the ecosystem and human health is difficult to assess. Not having an a priori understanding of the biological effects of these fluids leads to an open source of ongoing uncertainty in risk management. The overall goal of our research project is to identify chemical signatures and the mechanisms of toxicity of HFFF, thus, to develop assessment and monitoring tools for spills, thereby helping to define the biological zone of impact and improving risk assessment of HFFF. Here we present a case study of the complexity of these fluids and the challenges in assessing toxicity. To the best of our knowledge, this is one of the first toxicological studies on HFFF. An acute toxicity test was performed using zebrafish embryo, and EROD activity in zebrafish embryo exposed to HFFFs was also measured. The results indicated that the lethal toxicity might be attributed to organic contaminants and/or high salinity in HFFF, depending on the developmental stage of fish embryo. The induction of EROD activity in zebrafish embryo also suggested that polycyclic aromatic hydrocarbons might be one of the important contaminants group present in HFFFs, and CYP1A activity might be a good biomarker for rapid detection of HFFF spills in the aquatic environment.

Effects of water accommodated fractions of a hydraulic lubricating oil on larval amphibians (PL)

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Oil and gas transport depends on vast pipeline networks and many compressor stations, where automated actuator values pressurize and control the flow of the oil and gas. These valve actuators use hydraulic fluid as a lubricant, and leakage of the fluids has caused soil staining around the valves. Direct toxicity risks of this lubricating oil to aquatic organisms from groundwater infiltration as well as spring runoff (from snow melt and high rainfall) are uncertain. Therefore, we assessed the acute toxicity and sub-lethal effects of this lubricating oil UNIVIS J 13 on embryo-larval stages of two amphibian species, the African clawed frog (*Xenopus laevis*) and wood frog (*Lithobates sylvaticus*). Both species were exposed to a range of loadings of a water accommodated fraction (WAF) of UNIVIS J 13 for 96 hours. A dose-response relationship will be established and lethal loading 50 (LL_{50}) values will be determined. Surviving tadpoles were imaged to determine tail length, total length and snout-vent length, as well as assessed for morphological deformities. Response to exposure will be evaluated with quantitative polymerase chain reaction (qPCR) to examine expression of cytochrome P-4501A (CYP1A). The results will be used to determine and compare the sensitivity of a common laboratory frog species (*X. laevis*) and an environmentally relevant native frog species (*R. sylvatica*). The data gained from this study will be used to design and implement additional acute and sub-chronic toxicity tests involving exposure to UNIVIS J 13 on aquatic organisms to further investigate the potential risk to aquatic ecosystems from the release of lubricating oil.

Short-term bioaccumulation of hydrocarbons by the blue mussel (*Mytilus edulis*) exposed to conventional and non-conventional crude oils spilled under ice-covered seawater (PL)

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Exportation via Eastern Canada of crude oil extracted from the oil sands is on the rise. In fall 2014, the first Aframax medium-sized crude tankers loaded with diluted bitumen from Alberta, transported by train to a storage facility in Sorel-Tracy (Qc), were sailing on the St Lawrence river. If the Energy East pipeline project becomes a reality, more than 1 million of barrels per day of diluted bitumen will flow to refineries and port terminals in Quebec and New Brunswick. The risks of oil spill at sea will thus be increased throughout the year, including the winter season. However, very few studies report on the biological effects of non-conventional crude oil spilled in ice-covered seawater. Under these conditions of low water temperature (about -1°C) and ice cover, hydrocarbons solubility and evaporation should be limited whereas oil viscosity will increase. These factors should play a role on the extent of hydrocarbons bioaccumulation by a filter feeder such as the blue mussel (*Mytilus edulis*). Moreover, in very cold water with a low phytoplankton concentration, *M. edulis* can decrease its pumping rate by five and slow its metabolic activities. We measured the bioaccumulation of hydrocarbons (alkanes and polyaromatics) in blue mussels exposed for 48 hours to crude oils under controlled ice-covered seawater conditions, in an aerated 100 litre tank. One conventional crude oil (Heidrun) and two non-conventional crude oils (Access Western Blend and Cold Lake Blend in their winter formulation) were studied at 0.2, 0.4, 0.8, 1.6 and 3.2 $g\cdot L^{-1}$ nominal level of exposure. The water-soluble hydrocarbons were monitored by GC-MS analysis and by direct fluorescence measurement with a Turner Cyclops-7 submersible sensor for crude oil. Whole tissues concentrations of polycyclic aromatic hydrocarbons (PAHs, parents and alkyl derivatives) were quantified by GC-MS. The main observation is that mussels exposed to conventional crude oil accumulated ten times more PAHs (up to 36 $\mu g\cdot g^{-1}$ dry weight (dw), mainly alkyl derivatives) than mussels exposed to non-conventional oils (max 2.8 $\mu g\cdot g^{-1}$ dw for CLB and 1.1 $\mu g\cdot g^{-1}$ dw for AWB). This could translate to an inverse relationship between viscosity of the crude oil at 0°C and PAH bioaccumulation. Mortality (20%) was only observed for mussels exposed to Heidrun at 1.6 $g\cdot L^{-1}$. For none of the oil tested did the highest PAH concentration in tissues match the highest quantity of crude oil introduced under the ice layer. One possible explanation is that the diffusion of soluble hydrocarbons or small oil droplets in the water column (only mixed by air bubbling) was limited by the increased thickness of the crude oil layer under the ice.

Induction of EROD activity and oxidative stress in gill of rainbow trout (*Oncorhynchus mykiss*) exposed to hydraulic fracturing flowback fluid (PO)

Yuhe He[1], Erik Folkert[1], Shannon Flynn[1], Daniel Alessi[1], Jonathan Martin[1] and Greg Goss[1]

[1]University of Alberta

The emergence of hydraulic fracturing in oil and gas industry has made it necessary to query the potential toxicological and ecological risks this relatively new practice might pose to the environment. Although some attention has been given to the potential contamination of groundwater aquifers, knowledge of the entirety of the fracturing water cycle, in particular the surface-returned flowback fluid, is very limited. This hydraulic fracturing flowback fluid (HFFF) is a hyper saline mixture of fracturing proppants/surfactants containing numerous metals, radionuclides, polyaromatic hydrocarbons (PAHs), benzene and phenol derivatives. To date, significant knowledge gaps of the chemical and toxicological profile of HFFF poses extra difficulties for remediation efforts when spills and leaks occur. Additionally, governments and industry lack requirements and standards to which remediation efforts must be performed. Our program is trying to characterize the chemistry and toxicology of these HFFFs and to develop biomarkers and exposure limits, providing both governments and industry with feasible and realistic standards for monitoring and remediation. One of the objectives of our research project is to determine the nature of HFFF toxicity induced in fish and tissue locations where HFFF elicits its effects. This was done by performing sublethal exposure of HFFF to juvenile rainbow trout (RT) and measuring biochemical endpoints in various tissues. Ethoxyresorufin-O-deethylase (EROD) activity was measured as indicator of phase I enzyme activation, and thiobarbituric acid reactive substances (TBARS) were measured as indicator of oxidative stress generation. Exposure to HFFF resulted in significantly higher EROD activity and TBARS in RT gill. Removal of suspended particles from HFFF by filtration was able to attenuate/mitigate the effects, and activated charcoal treatment of HFFF was able to abolish the effects. The results indicated that exposure to HFFF induced phase I enzymes activation and generation of oxidative stress, and the organic components in HFFF might be the main contributors. In addition, compared to gill, the induction of EROD activity and TBARS was much lower, or even absent in RT liver at the same exposure time, indicating gill might be the first responding organ to HFFF exposure, at least in an acute exposure case. Further studies need to be performed to investigate the time course effects on induction of EROD and TBARS in different tissues.

Assessing the mechanistic toxicity of Oil Sands process affected waters (OSPW) (PO)

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Oil sands process-affected water (OSPW) produced during the extraction of bitumen in the surface-mining oil sands industry in Alberta, Canada, is acutely and chronicly toxic to aquatic organisms. It is known that the dissolved organic fraction of OSPW is responsible for most toxic effects, but knowledge of the specific mechanism(s) of toxicity is very limited. Using a bioassay effects-directed analysis approach, the dissolved organic fraction of OSPW was fractionated in three stages, with each stage involving identification of the toxic fraction by relevant toxicity screening such as the Microtox® assay and the 96-hour fathead minnow embryolethality assay. Chemical characterization was completed by use of reverse-phase high-pressure liquid chromatography (HPLC) in-line with a hybrid linear ion trap-orbitrap mass spectrometer. Relationships between the toxicity of each fraction, gene induction, and mechanism of toxicity were then examined by use of the Escherichia coli K-12 strain MG1655 gene reporter live cell array. This test system has been adapted to monitor the expression of greater than 1800 genes by use of transcriptional activity of the associated promoters coupled to a green fluorescent protein, facilitating the investigation of time-dependent changes in gene expression at high resolution. The tertiary fractions, F3-NE2a and F3-NE2b, had distinct chemical profiles, F3-NE2a contained predominantly naphthenic acids

(O_2^- species), while F3-NE2b contained mostly non-acid species (O, O_2, SO, NO). The tertiary fractions were acutely toxic to embryos of fathead minnow, and exhibited differing dose-response profiles. Effects on gene expression of the tertiary toxic fractions of OSPW were identified and used to construct a mechanistic profile of exposure to fractions of OSPW. Furthermore, this information was used to generate hypotheses for investigation *in vivo*.

Identification and evaluation of chemicals of potential concern to aquatic life in oil sands process-affected water from Base Mine Lake, Alberta (PO)

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Base Mine Lake (BML) is an end-pit artificial lake located within the Alberta oil sands area containing a mixture of fluid fine tailings, oil sands process-affected water (OSPW), and capped with a surface layer of fresh water. End-pit technology has been proposed as a method for long-term reclamation of oil sands activity; however, OSPW is a complex mixture of organics, metals, and salts, and is known to cause adverse effects in aquatic organisms. In order to properly understand the environmental risks and sustainability of end-pit lakes, it is necessary to be able to associate changes in the concentrations of specific chemicals of concern with changes in the toxicity of BML surface water to aquatic organisms. Conventional aquatic toxicity testing procedures used for regulatory compliance are often inadequate in assessing such complex mixtures, however, and are further confounded by the elevated salinity found within BML. Preliminary tests have shown that while BML OSPW is not acutely toxic to *Chironomus dilutus*, exposure to its salinity causes significant reductions in growth. Current work is focused on evaluating the effects of salinity on the toxicity of metals found within BML surface water to sensitive aquatic invertebrate species. The purpose of our research is to create a toxicity testing protocol specific to BML by evaluating aquatic test species that are tolerant of high salinity and which respond uniquely to the key chemicals of concern identified within BML OSPW.

Effect of dissolved organic compounds from oil sands process-affected water (OSPW) on toxicity of 7-isopropyl-1-methylphenanthrene (retene) to early life stages of Japanese medaka (*Oryzias latipes*) (PO)

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The composition of chemicals in tailings ponds that are used to store the oil sands process-affected water (OSPW) generated during the extraction of bitumen in Northern Alberta, Canada, is very complex. Among those chemicals that have the potential to cause toxicity are alkylated polycyclic aromatic hydrocarbons (alkyl-PAHs) that are associated with fine tailings, and a suite of oxygen (Ox), nitrogen (NO_X), and sulfur (SO_X) containing compounds that are dissolved in the aqueous phase. Although there are numerous studies of the toxicity of PAHs or the dissolved organic phase of OSPW to aquatic organisms, no study to date has investigated potential adverse effects of co-exposure to these chemical classes. In this study, effects of dissolved organic chemicals in relatively fresh OSPW from Base Mine Lake (BML-OSPW) and aged OSPW from the Pond 9 (P9-OSPW) experimental reclamation pond on the toxicity of 7-isopropyl-1-methylphenanthrene (retene) to early life stages of Japanese medaka (*Oryzias latipes*) were evaluated by use of a partition controlled delivery (PCD) method of exposure. Dissolved organic chemicals in OSPW were extracted without acidification by use of hydophilic-lipophic balance solid-phase extraction (HLB-SPE). Incidences of pericardial edema were greater in fish co-exposed to 15 $\mu g \cdot mL^{-1}$ of retene and BML-OSPW compared to fish exposed only to 1 or 15 $\mu g \cdot mL^{-1}$ of retene. In contrast, incidences of pericardial edema were not greater in fish co-exposed to 15 $\mu g \cdot mL^{-1}$ of retene and P9-OSPW. Expression of cyp1a was greater in fish co-exposed to retene and BML-OSPW compared to expression in fish exposed only to 1 or 15 $\mu g \cdot mL^{-1}$ of retene. Uptake of retene by fish exposed to BML-OSPW was greater compared to fish exposed to freshwater. Results suggest that the relatively fresh OSPW from Base Mine Lake can modulate toxicity of alkylated-PAHs to early life stages aquatic organisms, possibly by increasing their uptake.

Acute effects of exposure to oil sands-impacted sediments on the early life stages of *Xenopus laevis* (PO)

Melanie Gallant[1], Nicole Baldwin[1], Julie Bilodeau[2], Jules Blais[2], Bruce Pauli[3] and Natacha Hogan[1]

[1]University of Saskatchewan, [2]University of Ottawa, [3]Environment Canada

The Alberta oil sands contribute polycyclic aromatic compounds (PAC) to aquatic systems through release of waste from the extraction process, aerial deposition, landscape disturbance and natural geological formations. These pollutants have been observed in lakes, rivers, sediments, and snow cores in the Athabasca oil sands region. This is a concern given that PACs have been associated with toxicological effects in fish, particularly immunotoxicity. Amphibians also develop, reside and breed in oil sands impacted wetlands and are exposed to PACs throughout their lifecycle; however, the potential effects of PAC on amphibians are still unknown. In the present study, we assessed the embryotoxic potential to amphibians of sediments collected from the oil sands region; the sediments had a distinct and well-characterized PAC burden. *Xenopus laevis* embryos were exposed for 96 hours to sediment collected from two wetlands: one located near an oil sands extraction facility (impacted site) and one away from the surface mineable region (reference site). The sediments collected were characterized and analyzed for PAC content. Embryos were exposed in quadruplicate beakers containing 1 L of facility water and either 1 g or 5 g of sediment. Facility water alone served as a negative control. Sediment analysis revealed that the sum PAC was 1622.60 $ng \cdot g^{-1}$ wet weight (ww) sediment collected at the impacted site and 504.04 $ng \cdot g^{-1}$ ww sediment from the reference site. Following exposure, individual tadpoles were photographed, measured (tail length and snout-vent length (SVL)), and examined for developmental abnormalities. There was a significant increase in tail length, SVL and total length in tadpoles exposed to sediment from the impacted site with no increase in incidence of abnormalities. Expression of pro-inflammatory cytokines interleukin-1β (IL-1β) and tumor necrosis factor α (TNF-α) and interferon gamma (IFNγ), which are part of the innate immune response, were examined. IL-1β expression was increased in tadpoles exposed to sediment (5 g) from the impacted site with high PAC levels. The greater effects (in embryo-larval development and early inflammatory marker) observed with the impacted sediment are supported by the PAC concentrations analyzed in the sediments. These findings allow for better ecotoxicological characterization of sediments from oil sands impacted sites and provide data to assess the risk for amphibians developing in these systems with PAC-contaminated sediments.

Sediment and Soil Ecotoxicity

Broadening horizons: Investigating metal fate and effects in sediments (PL)

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Standard sediment toxicity tests are often conducted in isolation of techniques that can improve understanding of exposure. At CanmetMINING a weight of evidence approach has been employed alongside sediment toxicity tests to better understand the biological, chemical and physical influences affecting metal fate and effect in sediments. For example, mini-peepers, Diffusive Gradients in Thin films (DGTs), mineralogical characterization, sequential extractions and microbial assessments have been used to understand the various influences affecting metal fate and potential effects. A case-study investigating the bioavailability of selenium (Se) in sediments will be presented. Multiple techniques were incorporated into a 10-day *Chironomus dilutus* toxicity test which was conducted in both a static and flow-through exposure. The results from this study provided a better understanding of the bioaccumulation of Se in the benthic invertebrate and the drivers behind Se mobilization in sediment.

Validation of a new standardized test method for the freshwater amphipod, *Hyalella azteca* (PL)

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Environment Canada (EC) has been developing a method that includes a reproductive test endpoint as a complement to the existing 14-day standardized survival and growth test for *Hyalella azteca* (EPS 1/RM/33; 2013). The new methodology eliminates the potential for depuration and loss of sensitivity by conducting the entire exposure in sediment; in contrast to existing standardized methods (e.g., U.S. Environmental Protection Agency (USEPA), ASTM) where adults are transferred to a water-only exposure before release of their first brood at Day 28. Critical to the continuous sediment exposure method, procedures for the recovery of small amphipods from sediment after 42 days have been successfully developed and verified as demonstrated by acceptable variability and power analysis. Over the last three years, "proof of concept" experiments with the new continuous sediment exposure test method were successfully conducted using: 1) clean field-collected sediments considered ideal (i.e., containing a higher proportion of sand) and a more difficult sediment (i.e., with higher organic matter content, 2) a field-collected contaminated sediment, and 3) a clean field-collected sediment amended with silver nitrate ($AgNO_3$) and aged prior to organism exposure. In the contaminated sediment experiments (year

2), test sensitivity was investigated by comparing results of the draft EC 42-day reproduction test, the 42-day USEPA/ASTM reproduction test, and the 14-day Environment Canada (EPS 1/RM/33) survival and growth test. This year, the validation efforts with sediment spiked with $AgNO_3$ were challenging. In addition to measuring total and dissolved Ag in the bulk sediment and overlying water, silver (Ag) speciation (including Ag precipitation) was estimated using Visual MINTEQ (version 3.0). Biological measurements at the test end include the number of surviving adults (for both male and female), adult dry weight, and number of progeny. We also looked at Ag body burdens at test end. All tests were evaluated based on ability to meet proposed control validity/performance criteria, which included minimum percentage survival, mean final dry weight per adult, growth (increase in weight), and mean number of young per female. The performance of the validation trials will be presented.

Mechanisms affecting arsenic flux rates from prairie reservoir sediments, Buffalo Pound Lake, Saskatchewan, Canada (PL)

Lawrence D'Silva[1], Karsten Liber[1], Helen Baulch[1] and Lorne Doig[1]
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Buffalo Pound Lake, Saskatchewan, is a eutrophic reservoir that provides potable water to the nearby major cities of Regina and Moose Jaw. Lake sediments are hypothesized to be releasing nutrients into the overlying water and driving annual blooms, while simultaneously releasing associated arsenic (As). Although the magnitude of seasonal release of As from Buffalo Pound Lake sediment does not represent a human or animal health risk, it provides an opportunity to assess potential mechanisms of As release in prairie reservoir sediments. Using sediment core incubation experiments, this study investigated the effects of oxygen status and temperature on arsenic and phosphorous mobilization from Buffalo Pound Lake sediments. Sediment cores from four sites (cores collected June 2014) were incubated under either oxic or anoxic conditions for 35 days at 20°C, simulating summer conditions. Overlying water was analyzed for dissolved As, iron, manganese, and phosphorus fractions. A second core incubation experiment (cores collected October 2014) was conducted using a similar design with cores from only two of the sites incubated for 105 days at 4°C, simulating winter conditions. Flux rates for each treatment and site were calculated and statistically compared using two-way ANOVA. During the simulated summer incubation, As release did not significantly differ between oxic (0.61 ± 0.27 mg/m^2/day, n=12) and anoxic (0.73 ± 0.30 mg/m^2/day, n=12) treatments ($p > 0.05$). However, site location significantly affected As flux rate ($p < 0.001$). Arsenic remobilization under anoxic conditions was likely due to dissolution of sediment-associated iron and manganese oxyhydroxides, and release of associated As into overlying water. Arsenic remobilization under oxic conditions was likely a result of the formation of anoxic conditions below a thin oxic sediment-water interface, and the inability of this layer to scavenge As diffusing upward. Site differences in As release were likely due to differences in sediment geochemistry and As inventory, both of which are currently being assessed. During the simulated winter incubation, the concentrations of dissolved arsenic did not rise above background concentrations, regardless of oxygen treatment, and flux rates were not calculable. It is hypothesized that both microbial and macroinvertebrate activity are significantly inhibited at 4°C, both of which influence As flux and remobilization. Overall, the release rate of As from Buffalo Pound Lake sediments, for a given site, appears to be more dependent on temperature than oxygen status. Additional core incubations will be conducted to investigate the effects of alkaline pH waters and sediment microbial and macroinvertebrate activity on As remobilization and flux rates. *In situ* work will be completed to assess real-time As dynamics and to validate laboratory data.

Tesseracts and toxicity: Metal mixture testing in soil (PL)

Steven Siciliano[1], Beverley Hale[2], Mark Cousins[1], Kayode Jegede[1], Kobby Awuah[1] and Amanda Laird[2]

[1]University of Saskatchewan, [2]University of Guelph

Canada's mining industry is an important economic driver, employing over 300,000 Canadians and the largest private employer of aboriginal Canadians, contributing 3.5% of Canada's GDP. In Canada, the risk assessment for a site with multiple contaminants is based on the concentration of each contaminant of concern (CoC) relative to its toxicological reference value (TRV); if this ratio for each metal, termed a hazard quotient (HQ), is less than 1, then no adverse effect is expected. The expected toxicity of metal mixtures is not considered. The concentration addition (CA) theory of mixtures toxicity is that the effect of individual CoC is assumed to sum. We are assessing if CA is a valid theoretical model for the risk assessment of metal mixtures in soils when the concentration of metals in the mixture is elevated relative to the CCME's soil quality guidelines (SQGs). We will test the CA theory by developing toxicity thresholds for spiked metal mixtures for soil processes, collembolan, mites and enchytraeids across five different Canadian soils. These soils were selected to correspond to the soils used in the derivation of the European Union's Predicated No Effect Concentration (PNEC) model. In brief, the experimental approach is to spike the reference soils with single metals and metal mixtures of cobalt (Co), copper (Cu), nickel (Ni), lead (Pb) and zinc (Zn), from which dose-toxicity relationships will be determined for soil organisms. This project is testing the robustness of the CA assumption for soil risk assessment and offers predictive tools for sites with complex mixtures. If successful, this project will reduce the need for determination of site-specific remediation objectives by providing a robust process for determining site-specific estimates of metal mixture toxicity.

Managing contaminant risks to soil-associated flora and fauna in Canada: Evolution of standardized toxicity test methods in contrast with the retrogression of contaminated sites practitioners? (PL)

Doug Bright[1]
[1]Hemmera

The conceptual underpinnings, statutory frameworks, policies and guidance for the assessment and remediation (or risk management) of contaminated/brownfield sites in Canada emerged in the late 1980s through mid-1990s. Approximately two decades later, the current effectiveness of approaches and tools for managing the terrestrial ecological risks of soil contamination can be evaluated in the context of inter-generational change in the community of practitioners. This presentation discusses the evolution of ecological risk assessment approaches that underpin generic soil quality guidelines and standards in Canada and its provinces and territories, such as the Canadian Council of Ministers of the Environment (CCME) Soil Quality Guidelines, with a focus on direct contact toxicity potential for soil-associated flora and fauna. Considerable progress has been made since the development of the 1996 CCME Protocols for the Derivation of Canadian Soil Quality Guidelines, specifically with regard to (i) the development of standardized toxicity test methods for soil invertebrates and plants that better represent wildlands ecosystems in Canada, and (ii) approaches for the development of site-specific (Tier 2) soil remedial objectives. In spite of this, there is a troubling over-reliance on generic Canadian and provincial/territorial soil quality guidelines/standards that have a very large potential to either under- or over-predict risk potential for Canadian soil ecosystems that are substantially different than agronomic settings. The history and evolution of risk-based concepts for direct contact soil toxicity are discussed, and a case study is presented for a site in the Canadian high arctic with petroleum hydrocarbon contaminated soils to highlight the need for reduced reliance on use of generic direct contact soil quality guidelines and greater use of site-specific toxicity testing approaches.

Ecological description and classification of contaminated and reference soil samples sites (PL)

Jason Nelson[1], **Rick Scroggins**[2] **and Mary Moody**[3]
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During the development of ecologically relevant testing methods for the biological assessment of contamination, soil was collected from a wide range of forest and wetland ecosystems. During sample collection, considerable effort was made to characterize and understand the ecology of the sampling sites. Natural ecosystems are often non-homogenous, consisting of more than one vegetation community or soil type. Variability in such ecosystem components may affect contaminant distribution, concentrations and biological impact. Therefore, identification and description of major ecological variables and understanding of their spatial variability are necessary prerequisites for ensuring that samples are homogenous and representative, as well as ensuring that reference sites are well-matched with their contaminated counterparts. In practice, ecological characterizations of the sampling environment typically include standardized descriptions of the soil profile and plant community, and where available, classification of the local ecosystem using published ecological site or "ecosite" classification systems. Key considerations and practices important for soil toxicological test development will be discussed using examples from across the boreal and taiga regions of Canada.

A method for assessing contamination in boreal and northern wetland habitats using native plant species (PL)

Mary Moody[1] and Rick Scroggins[2]
[1]MJ Moody Environmental, [2]Environment Canada

Native plants can be used to assess the risks of contamination in both impacted and restored marshes, fens and bogs using newly developed wetland toxicity tests. Wetland plants are well suited to short-term tests and can serve as sensitive measures of risk and indicators of the success of restoration technologies. Over the past five years, tests have been developed for risk assessment and measurement of the effects of contaminants in impacted wetlands. The selection of ecologically-relevant plant species makes the tests relevant and valuable in the screening and assessment of vegetation in boreal and northern habitats. Test methods were developed beginning with 14 wetland plants native to marsh, fen and bog habitats of boreal regions. The short list of suitable plants was then refined to include four versatile and sensitive wetland species including bluejoint reedgrass, black spruce, Bebb's willow and sweet gale. All tests are performed over a short time (4-6 weeks) and are carried out at small-scale in growth chambers. Techniques for enhanced seed germination and seedling growth were developed, as some wetland plants require the use of young seedlings rather than seeds for test initiation. Results of case studies demonstrate that the ecologically-relevant test battery is sensitive and practical to use. The variable sensitivity of the wetland plants to brine was demonstrated as well as responses to weathered mixtures of petroleum hydrocarbons and metals. Each wetland plant test employs both substrate/soil and water from reference/background and contaminated areas, supporting a fuller understanding of the various modes of contact with receptors. Results of testing of different wetland contaminant situations will be presented and plans for final method standardization.

Development of a new Environment Canada test method for measuring survival and reproduction effects in soil using the oribatid mite, *Oppia nitens* (PL)

Rick Scroggins[1], Ryan Hennessy[2], Juliska Princz[2], Muriel Jatar[2], Heather Lemieux[2], Leana Van der Vliet[2] and Lisa Taylor[2]
[1]Ecotoxicology and Wildlife Health Division, [2]Environment Canada

In 1995, Environment Canada (EC) initiated a multi-year program to develop biological test methods that could be used to assess the toxicity of contaminants in soils, using terrestrial organisms. The goal was to develop test methods that were applicable to Canadian soil types using species that were representative of Canadian soil ecosystems. The creation of a new EC test method for measuring survival and reproduction effects in contaminated soil using an orbatid mite is the latest soil culture and methodology to be developed into a national standardized test. This toxicity test is based on EC research conducted by staff of the Soil Toxicology Laboratory. Scheduled round-robin testing and formal method writing will also be completed by BASS science staff. The principal test is a definitive 28-day test measuring chronic toxicity using adult survival and number of live juveniles as test endpoints. The mite species recommended as test organism of choice is *Oppia nitens*, a species found in surface soils across Canada. Key components of culturing, age synchronization and testing methodology will be discussed along with the planned inter-laboratory validation study. Recent data on test sensitivity will also be presented.

Can we predict polycyclic aromatic hydrocarbons bioavailability to mammals from incidentally ingested soils? (PL)

Steven Siciliano[1], Kyle James[1], Rachel Peters[1], Mark Cave[2], Mark Wickstrom[1] and Eric Lamb[1]
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Soil and dust ingestion is one of the major human exposure pathways to contaminated soil; however, pollutant transfer from ingested substances to humans cannot currently be confidently predicted. Soil polycyclic aromatic hydrocarbon (PAH) bioavailability is likely dependent upon properties linked to chemical potential and partitioning such as fugacity, fugacity capacity, soil organic carbon and partitioning to simulated intestinal fluids. We estimated the oral PAH bioavailability of 19 historically contaminated soils fed to juvenile swine. Between soils, fugacity does not predict PAH blood content, with the exception of benzo[a]pyrene. In contrast, between individual PAHs, fugacity predicts PAH blood content (Area under the Curve =0.33 log fugacity + 0.10, R^2=0.65, p<0.005, n=14). Soil fugacity capacity predicts PAH soil concentration with an average slope of 0.30 (μg PAH·g^{-1} soil) Pa-1 and R^2 values of 0.64-0.73. Because PAH blood content was independent of soil concentration, soil fugacity correlated to PAH bioavailability via soil fugacity's link to soil concentration. In conclusion, fugacity predicts PAH uptake from a soil into blood. However, something other than partitioning is critical

to explain the differences in PAH uptake into blood between soils. The kinetically constrained desorption of PAHs from carbonaceous geosorbents (i.e., black carbon), coupled with the short transit time in the gastro-intestinal tract, may explain differences between soils. Alternatively, soil, chyme and ileum biochemical interactions may be specific to each PAH, which would explain why fugacity predicts the blood uptake of some PAHs, such as benzo[a]pyrene, but not others.

New Tier 1 Boron Guideline for Alberta: Boron soil ecotoxicity and analytical methods development research (PL)

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Boron is an essential micronutrient for plants which occurs in soil and groundwater from a variety of natural or anthropogenic sources including agricultural and industrial applications. Boron can also be found in saline produced water and drilling waste, and is thus a common co-contaminant with salinity in Alberta. The existing Tier 1 Alberta soil guideline of 2 $mg{\cdot}kg^{-1}$ hot water soluble (HWS) boron is not based on a modern, risk-based approach but rather an older interim guideline. Various issues have been identified with this guideline in recent years, including evidence of high background levels above Tier 1 in many parts of western Canada, and that the HWS boron analytical method may not be suitable for evaluating toxicity. The interval between boron deficiency and toxicity is narrow for most plant species, and concentrations which may be toxic for one species may be deficient for another. Boron is adsorbed to soil organic matter and some mineral components such as clay, and thus the availability and toxicity is highly dependent on the specific physico-chemical properties of the soil. In addition to this, boron has relatively high solubility in water compared to many other metals and metalloids, thus making transport through groundwater to various receptors an important aspect of overall guideline development. An understanding of this transport requires an understanding of the adsorption properties of boron and how it varies in soil textures. Because of these factors, the analytical test method for boron must be able to accurately distinguish between deficient and toxic levels, and to provide reliable results over a wide variety of soil types and conditions. Substantial ecotoxicity research was performed with a set of ecologically and economically-important agricultural and boreal plant species, along with an earthworm chronic invertebrate toxicity test. Test soils included a wide range of Alberta-collected reference soils ranging from sand to sandy loam to clay loam to organic peat soils. Analysis of boron exposure concentrations in the toxicity tests included the traditional hot-water soluble boron method as well as the alternative saturated paste boron method to evaluate correlations between deficiency, toxicity, and measured soil boron concentrations. This presentation discusses the methods used and results obtained from these ecotoxicity tests, with results compared between the HWS *vs.* saturated paste methods across a range of plant species and soil types. Emphasis will be placed on our experiences with the new boreal regions ecotoxicity protocols. These results play a key role in the development of direct ecological soil-contact guidelines for boron, an important pathway for the development of updated Tier 1 boron guidelines.

Sensitivity of freshwater invertebrates to sediment-derived substituted phenylamine antioxidants (PO)

Ryan Prosser[1], Danielle Milani[1], Adrienne Bartlett[1], Patty Gillis[1] and Vimal Balakrishnan[1]
[1]Environment Canada

Substituted phenylamine antioxidants (SPAs) are produced in relatively large volumes and incorporated into a variety of consumer products (e.g., polymers, lubricants, dyes and adhesives). Based on their physical and chemical properties, SPAs could be potentially persistent, bioaccumulative and/or toxic; consequently, the Government of Canada is assessing this class of compounds under the Chemicals Management Plan for potential risks to the environment. The current study assessed the toxicity of four SPAs (diphenylamine; N-phenyl-1-naphthylamine; N-(1,3-dimethylbutyl)-N′-phenyl-1,4-phenylenediamine; and 4,4′-methylenebis[N-sec-butylaniline]) to three species of freshwater invertebrates: *Tubifex tubifex* (oligochaete worm), *Hyalella azteca* (amphipod), and juvenile and adult *Lampsilis siliquoidea* (freshwater mussel). Invertebrates were exposed to sediments artificially contaminated with individual SPAs for 28 days in aerated (static) vessels with a 3.5:1 water-to-sediment ratio. Mortality was assessed along with the following sublethal species-specific endpoints: reproduction (i.e., production of cocoons and juveniles), production of biomass, and rate of feeding for *T. tubifex*, *H. azteca*, and juvenile *L. siliquoidea*, respectively. In addition, reactive oxygen species, total glutathione, and lipid peroxidation in gill, digestive gland, and gonad of SPA-exposed adult mussels were measured to determine whether exposure to SPAs caused oxidative stress; bioaccumulation was also assessed in these tissues. A separate set of experiments was conducted to assess bioaccumulation in *T. tubifex*. Worms were exposed to sediment containing sublethal concentrations of each SPA for 28 days. Preliminary data analysis based on nominal concentrations shows that LC_{50}/EC_{50} values for all endpoints in the three species were more than 100 $\mu g \cdot g^{-1}$ sediment dry weight for all four SPAs examined. Analysis of tissue and sediment is ongoing; calculation of biota-sediment bioaccumulation factors will provide information on the bioavailability of SPAs to aquatic invertebrates. The results of this study will support environmental risk assessment activities to determine if SPAs could impact freshwater ecosystems.

Water Quality Benchmarks

Water Quality Guidelines in Canada: The meandering flow of the river! (PL)

Uwe Schneider[1]
[1]Water Quality in the 21st Century

My talk will follow the river of the Canadian Water Quality Guidelines (CWQGs) and will provide a glimpse at what could be encountered beyond the next bend. This river's journey has had some turbulent and some tranquil flows over the years. It is a long river, with rapids, falls, straight runs, and meandering bows along its path. Some crucial events have happened the last few years. As a result, the downstream future is open, and new directions can be envisioned. New riverine features could be created. Like an artificial lake, which acts as a national repository, comprising all the various national, provincial and site-specific benchmarks in Canada. Or a dredging and deepening of the old silted-up oxbows (i.e., the 1987 metal guidelines) in the upper reaches of the river. A cruise party on a floating steamboat (i.e., a workshop) could bring into focus new development vistas, may cause existing flow blockages to surface, drown misunderstandings, submerge problems, and throw overboard a lack of cooperation. Party boat guests would be water quality benchmark developers, evaluators and implementers. As well, a scientific expedition could dive into the river and explore the flow pattern of the riverbed (i.e., assess the current development protocol). And who knows what else we may encounter on our river journey?!

Using species sensitivity distributions to derive an arsenic water quality effects benchmark for aquatic ecological risk assessment (PL)

Kerrie Serben[1]**, James Dwyer**[1] **and Adrian deBruyn**[1]
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A site-specific water quality benchmark for arsenic was derived using a species sensitivity distribution (SSD) approach. The current Canadian water quality guideline for the protection of aquatic life is 5 micrograms per litre total arsenic, which was derived by multiplying the lowest acceptable toxicity endpoint by an arbitrary safety factor. Under the Canadian Council of Ministers of the Environment (CCME) guideline derivation protocol (2007), the safety factor approach is recommended for substances that either have inadequate or insufficient toxicity data for an SSD. Guidelines derived using an SSD are generally regarded as more robust than those based on the safety factor approach. We therefore reviewed available freshwater chronic toxicity studies and compiled relevant and reliable data based on a typical taxonomic composition for Canadian fresh waters, and found that there were enough data to meet the requirements of the SSD approach. Separate SSDs were derived for the two predominant forms of arsenic that occur in fresh waters, trivalent arsenic and pentavalent arsenic. We generally followed the data selection criteria and technical derivation steps outlined in CCME (2007), which allows some flexibility in benchmark derivations. We discuss where deviations from the process were warranted. When evaluated using SSDs, the 5% hazard concentration (HC_5) for pentavalent arsenic is lower than that for trivalent arsenic, indicating that pentavalent arsenic is the more toxic form. However, an even lower HC5 is obtained when all data (both pentavalent and trivalent arsenic) are combined. We explored the reasons for the differences among the SSDs and evaluated the sensitivity of the SSDs to inclusion or exclusion of data from some studies of uncertain reliability.

Field- and lab-based metal bioavailability and aquatic impact assessment at a closed gold mine (PL)

Helga Sonnenberg[1] and Karen Munro[1]
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Runoff and seepage from historical tailings at a remediated gold mine property in Geraldton, Ontario, enters Barton Bay (McLeod Tailings) and the Central Basin (Hardrock Tailings) of Kenogamisis Lake. Total arsenic in runoff ranges from 210 to 713 $\mu g \cdot L^{-1}$ for McLeod Tailings and 29.1 to 111 $\mu g \cdot L^{-1}$ for Hardrock Tailings. Corresponding lake values range from 5.9 to 93.2 $\mu g \cdot L^{-1}$ in Barton Bay and 3.3 to 41.8 $\mu g \cdot L^{-1}$ in Central Basin. Arsenic in lake water does not exceed the Provincial Water Quality Objective (PWQO, 100 $\mu g \cdot L^{-1}$) but does exceed the Interim PWQO and Canadian Water Quality Guideline for protection of freshwater aquatic life (CWQG-FAL) of 5 $\mu g \cdot L^{-1}$ in 86 to 100% of samples. Arsenic in sediment is also above the provincial severe effect level (33 $\mu g \cdot g^{-1}$). Mean arsenic content was 832 $\mu g \cdot g^{-1}$ in Barton Bay and 152 $\mu g \cdot g^{-1}$ in Central Basin. Are elevated metals in water or sediment causing adverse effects on resident aquatic organisms (phytoplankton, benthic invertebrates and fish)? How relevant are the guidelines, which differ by two orders of magnitude, in identifying aquatic effects? To answer these questions, an innovative study was undertaken using current field and laboratory based techniques. Four areas were sampled: two exposure areas (Barton Bay and Central Basin, adjacent to historical tailings areas) and two reference areas. Chronic toxicity tests using organisms from various trophic levels and ecological niches (*Chironomus dilutus*, *Hyalella azteca* and *Pseudokirchneriella subcapitata*) were conducted using site sediment and receiving water. Metal uptake in field and lab exposed invertebrates was analyzed and compared statistically between areas to Lethal Body Concentrations (LBC25s), and with the Metal Effects Addition Model (MEAM) to assess the metal mixture and bioaccumulation. Field studies included benthic community assessment following standard endpoints, metal bioaccumulation in *H. azteca*, and evaluation of Spottail Shiner and Walleye for growth, condition, and metal uptake in tissues. Arsenic speciation in Walleye tissue was also analyzed. Arsenic levels higher than water and sediment guidelines have led to bioaccumulation of arsenic in invertebrates and fish, but not to measurable adverse effects on phytoplankton, benthic invertebrates, or fish populations studied. This is likely related to the conservatism incorporated in sediment and water quality guidelines. A two order of magnitude difference in existing and Interim PWQOs makes the need for such a site-specific study essential to developing realistic toxicity thresholds and assessing potential cumulative effects of existing and future activities.

An evaluation of the effect of water hardness on chronic toxicity of cadmium in support of development of a Site-Specific Water Quality Objective (PL)

Brett Lucas[1], Ryan Hill[2], Emma Marcus[1], Karen Lee[1], Josh Baker[1], Yvonne Lam[1], Jesalin Wijaya[1] and James Elphick[1]
[1]Nautilus Environmental, [2]Azimuth Consulting Group

Chronic toxicity testing was performed using cadmium (Cd) across a range of water hardness concentrations (50, 100, 250 and/or 500 $mg{\cdot}L^{-1}$, as $CaCO_3$) relevant to a proposed mine using rainbow trout (*Oncorhynchus mykiss*; 70-day embryo-alevin-fry development test), *Hyalella azteca* (42-day survival, growth and reproduction test), and the cladocerans *Ceriodaphnia dubia* (7-day survival and reproduction test), *Daphnia magna* and *Daphnia pulex* (21-day survival and reproduction tests). Interestingly, and contrary to previous assumptions of cadmium toxicity, cladocerans exhibited no relationship between water hardness and cadmium toxicity in chronic exposures; for all three species, the most sensitive endpoint was determined to be reproduction. Rainbow trout and *H. azteca* both exhibited an inverse relationship between water hardness and cadmium toxicity; the slope of the relationship for rainbow trout was 0.707, which was similar to the pooled slope used in development of the British Columbia and Canadian Council of Ministers of the Environment (CCME) water quality guidelines for cadmium (i.e., 0.736 and 0.83, respectively). Conversely, *H. azteca*, which is among the most sensitive species to cadmium, produced a relatively shallow slope of 0.399, indicating a lesser effect of hardness on toxicity with this species than rainbow trout. The results from these tests were used to develop a site-specific water quality benchmark for the site.

Understanding the effects of sulfate and hardness in mine seepage on an aquatic receiving environment (PL)

Martin Davies[1], James Elphick[2], John Wilcockson[1] and Michelle DesJardins[3]
[1]Hatfield Consultants, [2]Nautilus Environmental Company Inc., [3]Copper Mountain Mine Ltd.

Sulfate is a common dissolved ion in aquatic environments that may occur at elevated concentrations in natural or mine-related seepages and industrial or agricultural waste waters. Advances in understanding the aquatic toxicity of sulfate recently led to the revision of the British Columbia guideline for protection of aquatic life, one of the few existing numerical guidelines for sulfate. This new guideline is novel in its consideration of hardness as a toxicity-modifying factor, recognizing that the aquatic toxicity of sulfate is reduced in high-hardness waters and adjusting the guideline from 128 $mg \cdot L^{-1}$ in soft waters to 429 $mg \cdot L^{-1}$ in hard waters. However, this guideline also recognizes potential adverse effects of high-hardness waters themselves, and limits the direct application of this guideline to many natural and anthropogenically affected drainage systems because elevated sulfate and hardness concentrations typically co-occur. In such cases, development of a site-specific sulfate benchmark is recommended. Wolfe Creek, located downstream of the Copper Mountain Mine near Princeton, British Columbia, has received mine seepage for many decades, with both sulfate and hardness concentrations sometimes reaching concentrations above 400 $mg \cdot L^{-1}$. A proposed science-based environmental benchmark for sulfate that is specifically protective of aquatic biota in Wolfe Creek was developed using a series of complementary laboratory- and field-based experiments, including assessment of rainbow trout (*Oncorhynchus mykiss*) egg-to-fry development using *in situ* hatchboxes and laboratory exposures, and additional acute and sublethal toxicity tests using invertebrate species (*C. dubia* and *H. azteca*). Laboratory exposures included Wolfe Creek water from upstream of the mine, laboratory water, undiluted mine seepage (788 to 947 $mg \cdot L^{-1}$ sulfate and 1,125 to 1,149 $mg \cdot L^{-1}$ hardness), mine seepage diluted using creek water, and mine seepage spiked with additional sulfate (as Na_2SO_4 or $CaSO_4/MgSO_4$) to approximately 1,800 $mg \cdot L^{-1}$. These experimental studies were supported by comprehensive fish-population, periphyton and invertebrate-community surveys upstream and downstream of affected creek reaches. No adverse effect of undiluted or sulfate-spiked mine seepage was observed on larval trout survival or growth rates in laboratory or *in situ* hatchbox exposures (i.e., no effect up to 1,978 $mg \cdot L^{-1}$ sulfate and 1,130 $mg \cdot L^{-1}$ hardness), or on invertebrate survival in laboratory exposures. However, undiluted mine seepage and seepage spiked with additional sulfate up to 1,870 $mg \cdot L^{-1}$ sulfate (up to 2,020 $mg \cdot L^{-1}$ hardness) affected *C. dubia* reproduction and *H. azteca* growth, with derived EC_{20} values of 833 and 772 $mg \cdot L^{-1}$ sulfate, respectively. Results suggested no current effect of sulfate or hardness in mine seepage on the aquatic biota of Wolfe Creek, consistent with ongoing, field-based biological monitoring studies. Based on these results, a science-based environmental benchmark of 567 $mg \cdot L^{-1}$ sulfate in Wolfe Creek was proposed.

Environmental Assessment and Cumulative Effects

Using Landsat imagery to backcast fire and post-fire residuals in the Boreal Shield of Saskatchewan: Implications for woodland caribou management (PL)

John Kansas[1], Javier Vargas[2], Hans Skatter[3], Brady Balicki[4] and Kevin McCullum[5]
[1]Kansas & Associates, [2]Ecotone Environmental, [3]Omnia Ecological Services, [4]Cameco Corporation, [5]Saskatchewan Ministry of Environment

The woodland caribou (*Rangifer tarandus*) is designated as threatened under Canada's *Species at Risk Act*. A 2012 Recovery Strategy for the boreal population of caribou identified critical habitat for all but one of 51 caribou ranges in Canada – Saskatchewan's Boreal Shield (SK1). The strategy identified 65% undisturbed habitat as the threshold below which a local population was not likely to be self-sustaining. Disturbance was measured as the combined effects of fires <40 years and anthropogenic land use. The fire component of the total disturbance model used existing fire polygons that were delineated using traditional mapping methods. Our study maps fire events from 1988 to 2013 for 66,500 km^2 of the SK1 range using dNBR analysis of Landsat-TM imagery. Annual fire event areas were similar between manual and Landsat-derived burn perimeters but the manual methods significantly overestimated burned land area by ignoring post-fire residuals and water bodies. The federal recovery model assumes that all lands within provincial fire polygons are inadequate as caribou habitat, and ignores the value of post-fire residuals and water bodies as potential habitat for foraging, movement, calving and predator avoidance. For northern Saskatchewan, where disturbance thresholds have yet to be determined, this refined mapping allows for spatial tracking of undisturbed habitat at a finer resolution than the coarse fire mapping and will better serve caribou management initiatives.

Refining environmental impact assessments: Pathway analysis (PL)

Jennifer Fernet[1]
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Approaches to environmental impact assessment (EIA) are constantly evolving and being refined. Typically the EIA considers a number of elements, including issue scoping, selection of valued components (VCs), baseline studies, pathways analysis, residual effects analysis, determination of significance, and recommended monitoring and follow-up programs. The approach involves a systematic consideration of how Project activities may interact with the environment and result in an effect on one or more environmental components. Golder Associates Ltd. have used a pathways analysis to focus the EIA and it is an important component in the success of our EIA process. The overall goal of the EIA is to objectively and transparently present a comprehensive assessment of the potential environmental effects of a project on VCs. The intent of the

pathways analysis or screening of project interactions is to focus the assessment on those interactions with the greatest potential to result in significant effects to both the biophysical and socio-economic components of the environment. Pathways are determined to be primary, secondary or no-linkage using scientific, local and traditional knowledge, logic, experience with similar developments, and environmental design features and mitigation. Primary pathways require further evaluation through more detailed quantitative and/or qualitative residual effects analyses and classification to determine the environmental significance of a project effects on VCs. My presentation will provide an overview of our approaches and methods for the pathway analysis. Topics covered will include definitions of VCs, assessment endpoints and measurement indicators, and pathways analysis. Specific examples from recent environmental assessments will be provided.

Analysis of cumulative effects and determination of significance (PL)

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The addition of any one project seldom results in significant residual effects to value components (VCs) of the biophysical, cultural and socio-economic environments. To sufficiently understand the contribution of a project to effects on the social-ecological system the analysis of cumulative effects is a necessary element of environmental impact assessment. Cumulative effects represent the sum of all natural and human-related influences on the VCs of biophysical, cultural, social and economic environments through time and across space. Not every VC requires an analysis of cumulative effects. The key is to determine if the residual effects from a project and previous, existing and reasonably foreseeable developments overlap or interact with the spatial distribution of the VC. Therefore, the temporal and spatial boundaries of the analysis and assessment of the significance of the incremental and cumulative effects from a project and other developments is specific to the geographic movement and distribution of each VC. The practice of environmental assessment has been quickly evolving during the past 10 years. Golder Associates Ltd. has been on the leading edge of these changes with our work for clients in British Columbia, Saskatchewan, Ontario, the Northwest Territories and Nunavut. My presentation will provide an overview of our approaches and methods for analyzing and assessing the significance of ecological cumulative effects. Many of the principles and concepts are in the current draft assessment guidelines being developed by the Canadian Environmental Assessment Agency. Topics covered will include definitions of VCs, assessment endpoints and measurement indicators, spatial and temporal boundaries, residual effects characterization and determining ecological significance. Specific examples from recent environmental assessments will be provided.

The effect of historical discharge from the abandoned Giant Mine on slimy sculpin (PL)

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The Giant Mine is an abandoned gold mine located in Yellowknife, NWT. Previous studies have shown elevated levels of arsenic in the water, sediment, fish and benthic biota of Yellowknife Bay and nearby aquatic ecosystems. To increase understanding of current conditions, slimy sculpin (*Cottus cognatus*) length, weight, relative weight and whole-body arsenic content were determined for fish collected from the Yellowknife River (upstream reference), and four areas in Yellowknife Bay, including Baker Creek Outlet (exposure), Back Bay (near field), Mosher Island (far field) and Horseshoe Island (downstream reference). Data were analyzed using a series of Two-Sample Kolmogorov-Smirnov Tests for length distribution, and one-way ANOVA coupled with Critical Effect Size (CES) for other endpoints. The analyses were consistent with, and tested, the current and proposed non-lethal sampling and analytical methods as outlined for the Canadian Environmental Effects Monitoring program. Slimy sculpin were found to be heavily contaminated with arsenic, depending on environmental exposure. It was noteworthy, however, that despite decades of exposure to mine discharge, slimy sculpin were collected at all sites within the study area. There were significant between-site differences in length distribution, with reference site communities of slimy sculpin containing a greater proportion of larger, older fish compared with exposure sites. There were significant differences in young-of-year length, although all differences were considerably less than the CES. These results were considered artifacts of the large sample size and therefore not environmentally significant. There were also significant differences in relative weight, although these were greater than CES. The relative weight data were complicated by a seasonably variable sampling program, and highlighted the importance of consistent sampling at an appropriate time of year for non-lethal monitoring programs. The results indicated that slimy sculpin populations differed, depending on proximity to the Giant Mine discharge. The study also confirmed the utility of a non-lethal sampling approach, although with some caveats.

Investigation of Cause: Tools for assessing habitat quality *vs.* metal exposure using a weight of evidence approach (PL)

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Since 2002, all metal mines in Canada have been required to conduct Environmental Effects Monitoring (EEM) studies under Schedule 5 of the federal *Metal Mining Effluent Regulations* (MMER, 2002). Mines are strongly encouraged to follow Environment Canada's Technical Guidance, which is relatively prescriptive for the first three phases of the program: Initial, Periodic and Focused (magnitude and extent) Monitoring. For the subsequent Investigation of Cause (IOC) phase, very little guidance has been provided. Within the EEM program an "effect" on the fish or benthic invertebrate community is defined as "a statistical difference between exposure and reference area", with little regard to the biological significance of these effects, and in some cases, statistically significant effects have been observed between reference areas. Therefore, there was a need to distinguish between true mine-related effects versus differences in habitat quality among sampling areas. Standard EEM approaches for evaluating fish community health and habitat quality were applied to biological communities downstream of a base metal mine discharge in northern Manitoba. Effects on the benthic invertebrate community suggested that the exposure area was healthier than the reference area (greater density, evenness and taxa richness). These results were supported by the small-bodied fish results which showed consistent stimulatory effects (increased reproduction, condition and energy storage) in Brook Stickleback from the exposure area. Inconsistent effects were observed in the large-bodied fish communities. Increased growth and condition (stimulatory effects) as well as decreased survival and reproduction (inhibitory effects) were observed in the yellow perch (*Perca flavescens)* communities. Results of previous EEM studies suggest that exposure fish were fatter, younger fish with less predation pressure than the reference area fish and the cause of effects has not been clearly linked to mine effluent. A weight of evidence approach was used to assess whether biological effects were due to direct metal exposure or strictly to habitat quality differences between areas. Total metal burdens in fish livers and gonads as well as total liver metallothionein concentrations were analyzed to assess direct metal exposure in fish from both areas. Bioenergetics (triglycerides, glycogen, NDPK, condition, LSI, GSI), benthic invertebrate community assessments, and trophic biomass studies—including an assessment of caloric content of each trophic level in conjunction with a fish stomach content analysis and macrophyte survey—were conducted as an assessment of habitat quality differences (food resource availability, food preference and food quality) among areas. Supporting water and sediment quality (both metals and nutrients) were also examined.

Peel River watershed: Temporal trends in hydrology and water chemistry (PL)

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The Peel River watershed is located in the Yukon Territories and is an almost pristine wilderness, although it is currently under pressure from resource industries. The objectives of this study were to define the seasonal hydrological cycle within the watershed, to develop an understanding of baseline water chemistry, to determine and describe any seasonal differences in water chemistry, to determine spatial variability in water chemistry, particularly moving downstream, and to determine and describe any temporal trends in water chemistry and/or hydrology. To address these objectives, several comprehensive datasets were provided, including a hydrometric dataset of flow in the Peel River collected from 1969 to 2010, a set of water chemistry data collected at a downstream site from 1969 to the present, a set of water chemistry data collected at various sites in the Peel River watershed between 2002 and 2007, and a limited set of sediment chemistry data collected between 2002 and 2007 from one downstream site. The Peel River and its tributaries are alkaline (pH=7.9-8.4) with total dissolved solids ranging from 170 to 300 $mg \cdot L^{-1}$. Calcium, magnesium, bicarbonate and sulfate are the dominant major ions in the Peel River watershed. Flow rate in the Peel River varies seasonally, and there are consistent seasonal differences in concentration related to flow rate: concentrations of dissolved substances decrease during spring freshet, increase during recession of flow through the summer and are maximal during winter base flow. The opposite pattern is observed for particulate materials, and for metals that are strongly and positively correlated with turbidity. The watershed is currently undergoing changes both to water quantity and quality. Over the past 40 years, the total yearly flow has not changed, but the onset of winter base-flow has occurred later in the year, and the amount of flow in the winter has almost doubled. During the same time period, the major ions calcium, magnesium, and sulfate have increased in concentration during all seasons of the year. These trends suggest that the watershed is less "frozen" than it used to be, and that relatively "unweathered" material has become increasingly exposed to runoff over time.

Future directions for using natural variability to develop triggers for designing and adapting environmental monitoring programs for cumulative effects assessment (PL)

Kelly Munkittrick[1], Tim Arciszewski[1], Bruce Kilgour[2], Keith Somers[3] and Tim Barrett[4]

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A big challenge in biological monitoring is how to incorporate natural variability in biological endpoints into decision-making. As the temporal and spatial scales of monitoring increase in long-term programs, available statistical power increases the potential for identifying very small changes. There are huge costs and risks to management decisions based on small biological changes which may be ecologically meaningless, or for which there is an insufficient historical baseline data to inform decision-making. We have identified a conceptual model for a scalable adaptive monitoring framework that defines changes using a normal or expected range for biological data, based on measures of natural variability at multiple spatial and temporal scales. The estimates of the normal range limits are dependent on the quantity of data, improve over time, and stabilize around 2 standard deviations when adequate baseline data are available. This adaptive monitoring framework can be used to identify triggers to define when local change is of sufficient concern that it needs focused attention, and allows the prioritization of resources to areas with the most uncertainty or change.

Incorporating the duty to consult and traditional knowledge into environmental assessments (PO)

Katie Zdunich[1], Brent Murphy[1] and Phil Anaquod[1]
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In Canada, the Supreme Court of Canada has ruled that the Crown has a legal duty to consult with and, where appropriate, accommodate, Aboriginal communities on matters that can affect Aboriginal claims or rights. Although it is the government's responsibility to implement and carry out the duty to consult when it is required, often the proponent is in the best position to provide First Nations and Métis communities with project-specific details. The objective of our poster presentation is to show project examples of how a proponent can carry out duty to consult activities and how they can work with Aboriginal communities to consider the traditional knowledge each community has of the project area and identify the necessary Valued Ecological Components (VECs) for the environmental assessment.

Environmental Assessment, Protection and Management for Uranium Mining and Milling

Environmental performance of a theoretical modern uranium mine/mill: Based on a 13-year monitoring dataset (PL)

Malcolm McKee[1], Elias Dagher[1], Nana-Owusua Kwamena[1] and Barbara Dowsley[1]
[1]Canadian Nuclear Safety Commission

Uranium mine and milling operations are required to design, implement, interpret and maintain extensive environmental monitoring programs. The results of environmental monitoring programs are contained in various compliance documents required for submission to provincial and federal regulators. The results have also been incorporated into a number of comprehensive environmental assessments under the *Canadian Environmental Assessment Act*, the Nunavut Impact Review Board and the Comité d'examen des répercussions sur l'environnement et le milieu social (COMEX), all of which have been extensively reviewed by specialists in multiple government agencies. Despite these being public documents, the information on the environmental performance of modern Canadian uranium mines is lacking in the peer reviewed scientific literature and formal government publications. This results in the over-representation of environmental conditions associated with historical poor mining practices in the scientific literature by those either unaware of or unwilling to use the modern dataset. The Canadian Nuclear Safety Commission (CNSC) presented a 13-year (2000-2012) environmental monitoring dataset to demonstrate, in a public friendly manner, the potential environmental performance one might expect from a modern uranium mine/mill regulated by the CNSC during the recent Quebec Bureau d'audiences publiques sur l'environnement (BAPE) environmental assessment hearings on "Issues Related to the Uranium Mining Industry". Thirteen years of environmental data (2000-2012) from four operating northern Saskatchewan uranium mining and/or milling sites were incorporated into one database, thereby capturing a range of site-specific environmental performance factors. The focus was on the core monitoring elements common to all the operating uranium mines and mills: air, surface water, fish and aquatic sediments, addressing up to 22 contaminants of potential concern (COPCs). The mean concentrations of nuclear and hazardous substances were presented relative to their distance from their primary source of release to the environment. Based on existing lease surface areas, monitoring stations less than 2 km from the source were classified as "onsite", those beyond as "offsite". The concentrations were compared to applicable guidelines and criteria for the protection of human health and the environment and to the range of natural background concentrations from the region. Radiological doses were calculated and compared to reference doses derived from the CNSC public dose limit of 1 mSv per year above natural background radiation. The results demonstrate that modern uranium mining and/or milling can be safely executed in order to ensure the protection of public health and the environment.

The evolution and contribution of environmental performance reporting for uranium mines and mills in Saskatchewan (PL)

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Uranium mines and mills in Saskatchewan are regulated under the auspices of both the Governments of Canada and Saskatchewan. Federally, uranium mines and mills are regulated under the *Nuclear Safety and Control Act* and must meet reporting requirements of the Canadian Nuclear Safety Commission (CNSC). Provincially, they are regulated under *The Environmental Management and Protection Act*, 2010, among others. The Saskatchewan government requires that uranium mines and mills submit an environmental performance report (EPR) every five years. The five-year cycle for EPRs ensures that regular updates are provided on current environmental performance compared to model predictions to verify compliance, analyze environmental trends and evaluate the effectiveness of environmental monitoring and management. This cyclical approach was instrumental in the adoption of site-specific environmental risk assessments (ERAs) as living or evergreen documents in the CNSC Environmental Protection Framework. We will discuss lessons learned when preparing EPRs, the value of investing in an evergreen ERA, and our living model, IMPACT, as a tool to foster understanding of the receiving environment and inform the licensing process.

Potential impacts following an accidental release during transportation (PL)

Stacey Fernandes[1], Mehran Monabbati[1], Dale Huffman[2] and Sarah Benson[2]
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Uranium ore concentrates (UOC), commonly referred to as yellowcake, produced in Northern Saskatchewan are packaged in 205-L steel drums, loaded in sea-cans or transport trailers, and transported to their destination by road and rail. An assessment was conducted to examine the potential risk and consequences for the transportation of UOC in Canada via ground transportation routes. The results of this assessment can be used for planning purposes and to inform emergency response plans. As the assessment covered a large range of potential releases and receiving environments, different bounding scenarios were identified and carried through a consequence assessment. In the presentation, the approach for the consequence portion of the assessment for the aquatic environment will be discussed. A range of receptors was selected including fish and benthic invertebrates and waterfowl that can reside in the area. The consequence assessment included consideration of the impact of a short-term release followed by a longer duration impact from residual contamination that may remain after remediation efforts.

Effects of uranium mining on benthic invertebrate communities in the Athabasca Basin of Northern Saskatchewan (PL)

Bruce Kilgour[1], Barbara Dowsley[2], Malcolm McKee[2] and Steve Mihok[2]
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Aquatic environments downstream of uranium operations (mining/milling) in Northern Saskatchewan are exposed to a variety of chemical and physical disturbances. There is extensive regulatory documentation of monitoring and effects assessments for modern uranium operations, though little is available in the scientific literature. The Reference Condition Approach was used to predict expected benthic invertebrate communities for unexposed and exposed lakes. This approach was used to identify impacted communities that differed significantly from expected natural regional compositions. The number of taxa (taxa richness) downstream of approximately half of the uranium operations effluent release points was lower compared to richness observed in reference conditions. Richness was lower in lakes with higher concentrations of metals and radionuclides in the sediments.

Investigation of cause for a Northern Saskatchewan mine (PL)

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Environmental Effects Monitoring (EEM) activities performed at a mine under development in northern Saskatchewan have detected changes in the benthic invertebrate community and fish population characteristics in receiving environments, despite the fact that water chemistry has remained within water quality guidelines. Observed effects have been stimulatory in nature and include an increase in richness and density of invertebrates and an increase in male size-at-age, weight, and female liver weight relative to body weight of slimy sculpins. An Investigation of Cause (IOC) was performed, utilizing laboratory toxicity tests, field measurements of habitat, and a detailed review of existing data to identify the cause of these apparent effects. For invertebrate populations, an increase in ionic strength in the receiving environment associated with the discharge, from very low ionic strength background conditions, was identified as the cause of increased richness and density. For slimy sculpins (*Cottus cognatus)*, the effects appear to have been caused by differences in habitat and associated prey at the exposure and reference sites; in particular, *Gammarus* were present in significant quantities in the gut at the exposure site, and not the reference area; this effect was likely unrelated to the treated effluent.

Factors influencing polonium-210 bioaccumulation in resident biota and caged oligochaetes in receiving environments downstream of closed uranium mines (PL)

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Polonium-210 (^{210}Po) activity in resident biota was investigated for evidence of food web transfer to higher trophic levels (using δ^{15}N isotope analysis) in a lake downstream of a decommissioned uranium mine in Ontario, Canada. Top trophic level organisms such as lake trout and white sucker showed decreased ^{210}Po activities in muscle compared to the majority of probable prey items. On the basis of the ^{210}Po activities in organisms from different trophic levels of the same food web there was no evidence for ^{210}Po biomagnification in this freshwater lake. In addition ^{210}Po bioaccumulation from sediment and water was investigated using caged oligochaetes (*Lumbriculus variegatus*) deployed in five areas downstream of decommissioned uranium operations in Ontario and Saskatchewan, Canada. ^{210}Po accumulation factors from sediment and water to oligochaete tissue were used as a method to investigate variables that influence ^{210}Po bioavailability to these organisms. ^{210}Po was found to be predominantly associated with sediment, and sediment mineralogy was the major influence on ^{210}Po bioavailability. The source of ^{210}Po in the environment was through the radioactive decay of radium-226 (^{226}Ra), a longer-lived pre-cursor radionuclide in the ^{238}U decay series. It was hypothesized that ^{210}Po retains some ^{226}Ra-based mineral associations, after ^{226}Ra has decayed, depending on the type of mineral association that ^{226}Ra had (i.e., co-precipitated or surficially adsorbed).

Effects of chronic exposure to radium-226 on health and reproductive fitness of mice (PL)

Marilyne Stuart[1], Stephanie Walsh[1], Meloja Satkunam[1,2], Ben Su[1,2], Amy Festarini[1], Michelle Bugden[1], Harry Peery[1] and Carmel Mothersill[2]
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The purpose of the study conducted at Canadian Nuclear Laboratories was to assess health, growth and reproductive success of mammals continuously exposed, for multiple generations, to radium-226 (^{226}Ra). Mice of the CBA/CaJ strain were exposed via drinking water to 0.012, 0.076, 0.78 and 8.0 $\text{Bq}\cdot\text{L}^{-1}$ of ^{226}Ra. The exposure commenced two weeks prior to breeding of the parent generation. Breeding was at age 8 weeks (8-10 weeks for the parent generation). Exposures of breeding mice were maintained through gestation periods of the parent generation and through growth and gestation of the subsequent three generations. Reproductive fitness (using pregnancy rates and number of weaned pups per litter as indicators), growth (evaluated using weight) and physical condition (assessed based on the frequency of malformations, abnormal behaviours, sickness including the development of neoplasms, and mortality)

of pups were not negatively affected by their own, their parents' and grandparents' constant (including in utero) exposure to low levels of ^{226}Ra compared to the corresponding control mice. Our results from the murine model suggest that multi-generation chronic exposure to ^{226}Ra at environmental levels found at contaminated mining sites may have no significant adverse effects on reproduction in small mammals. Tissues collected during the study are currently being used to obtain dosimetric data and to measure a number of cellular and molecular biological markers of radiation exposure and effects.

Overview of a country foods study conducted near the decommissioned Beaverlodge uranium mine and mill site in northern Saskatchewan (PL)

Samantha Pham[1] and Ryan Froess[1]
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The decommissioned Beaverlodge uranium mine/mill site is located approximately 8 km east of Uranium City in northern Saskatchewan. The mine operated for almost 30 years between 1953 and 1982. Decommissioning of the site occurred from 1983 to 1985, and thus, the property is currently in its 30th year of transition phase monitoring. During the public meetings in Uranium City, local residents raised concerns about the potential health risks associated with traditional harvesting of local food sources or country foods. In response to these concerns, Cameco retained Canada North Environmental Services to complete a two-year long Uranium City Country Foods Study. The study examined the country food consumption patterns of residents in the Uranium City area and measured potential contaminant levels in widely consumed country foods. The first portion of the study involved conducting interviews with Uranium City area residents to identify the type, amount, frequency, and location of traditionally harvested foods. The interviews were completed by a trained local resident and included a total of 115 people representing approximately 91% of year-round and seasonal residents of Uranium City. The results provide information on the types of mammals, birds, fish, berries, and medicinal plants most heavily utilized by the residents as well as the approximate hunting and gathering locations. A review of existing data, along with interview results from the Year 1 study, was completed to determine which types of country foods should be sampled and obtained from local residents. The second portion of the study included the collection and chemical analysis of different types of country foods considered essential to the residents of Uranium City. The analysis was performed to gain a better understanding of the potential human health implications from the consumption of country foods. The information gathered during the Uranium City Country Foods Study was used to complete a human health risk evaluation for the Beaverlodge area. The results of the risk evaluation will be used to help assess the need for additional remediation on the Beaverlodge properties.

Risk evaluation of the country foods study conducted near the decommissioned Beaverlodge uranium mine and mill site in northern Saskatchewan (PL)

Harriet Phillips[1], Leah Leon[1], Stacey Fernandes[1] and Mike Webster[2]
[1]ARCADIS Canada Inc., [2]Cameco Corporation

The Beaverlodge Mine in Northern Saskatchewan ceased operation in 1982 and underwent decommissioning between 1983 and 1985. Residents of the nearby Uranium City have raised concerns about the potential health risks associated with traditional harvesting of country foods from local sources. In response to these concerns, Cameco initiated a two-year long Uranium City Country Foods Study. The first part of the study was carried out by Canada North Environmental Services and examined the country food consumption patterns of the residents of the Uranium City area and measured potential contaminant levels in widely consumed foods. The second part of the study was carried out by ARCADIS Canada Inc. (formerly SENES Consultants Ltd.) and involved the incorporation of the data collected in part 1 into a risk evaluation for exposure of Uranium City residents to constituents in country foods obtained from Camsell Portage, Uranium City and the Beaverlodge Properties. Uranium City is a small community with a year-round population of approximately 70-100 people. The evaluation considered exposure of both child and adult residents via ingestion of country and non-country foods, and compared the exposure to that of typical Canadians who are exposed from consumption of supermarket foods. The exposure from ingestion of food was also compared to toxicity reference values (TRVs), which are levels of exposure that are likely to result in no appreciable risks or measurable adverse effects to human health. Receptor characteristics (e.g., foods consumed, harvest locations, etc.) and exposure pathways (e.g., inhalation, ingestion, etc.) were taken into consideration. Thus, country food intake rates developed from the information collected during the Year 1 Study and measured concentrations of Constituents of Potential Concern (COPC) in the country foods collected during the Year 2 Study were used in the evaluation. The evaluation considered exposure under three different food collection scenarios and also evaluated both maximum and mean exposures to non-radionuclides and radionuclides. The presentation will discuss the risk evaluation results.

Bioavailability of sediment-associated uranium to the freshwater midge, *Chironomus dilutus*: Lessons learned from sediment physicochemical properties (PO)

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Uranium (U) can enter the environment from mining and milling of U, often accumulating in sediments to concentrations that have the potential to adversely affect benthic organisms. Thus, predicting the sorption, mobility, and fate of U in the environment is important for our understanding and assessment of U-contaminated sites. Physicochemical properties of sediment, such as particle size and total organic carbon (TOC) content, have been shown to modify bioavailability and toxicity of U in freshwater environments. Sorption of U is strongly influenced by pH, but few studies have investigated this effect in conjunction with physicochemical properties of sediment. The goal of this research was to quantify the role different sediment physicochemical characteristics have on altering U sorption and U bioaccumulation by *Chironomus dilutus* in a number of field sediment ranging in sediment properties (pH, organic matter, particle size, cation exchange and iron oxide). Sorption of U was quantified using Organisation for Economic Co-operation and Development (OECD) equilibrium batch experiment guidelines. Test solutions were reconstituted to mimic water quality characteristics commonly found downstream of Saskatchewan U mines. Bioaccumulation of U by *C. dilutus* larvae was investigated using 10-day sediment toxicity tests with 18 U-spiked field sediments with a wide range of physicochemical characteristics collected from sites around U mines in northern Saskatchewan, Canada. U concentrations tested were environmentally relevant in both the sorption tests (0.023 to 2.3 mg $U \cdot L^{-1}$) and the U-spiked field sediment tests (50 and 500 mg $U \cdot kg^{-1}$ dry weight (dw)). The degree of adsorption to each sediment was strongly pH-dependent, increasing sharply around a pH of 6, and then decreasingly significantly over the pH range of 7 to 8. There were significant differences in the pH-dependent sorption among different sediment physicochemical characteristics, with the greatest adsorption occurring in sediments with approximately 37% fine fraction (clay + silt) and 12% TOC content. This trend coincides with maximum reduction in U accumulation by *C. dilutus* larvae from U-spiked sediment bioaccumulation studies. U concentrations accumulated by *C. dilutus* larvae varied 26-fold (5 to 132 $mg \cdot kg^{-1}$ dry weight (dw)) and 23-fold (20 to 452 $mg \cdot kg^{-1}$ dw) among the different sediments spiked with 50 and 500 mg $U \cdot kg^{-1}$ dw, respectively. Thus, whole-sediment U concentrations were a poor predictor of U bioaccumulation and bioavailability and instead, significant negative relationships were observed between U bioaccumulation and sediment properties: cation exchange capacity (CEC), iron oxides (FeO_x), TOC and fine fraction. Results from this research improve our understanding of U sorption in natural field sediment, and can be used to quantify sediment properties that will modify site-specific ecological risks at sites with U-contaminated sediments.

A spatial and epidemiological analysis of deformed amphibians in southern Québec, Canada: What do deformed frogs tell us about the state of the environment? (PL)

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[1]Environment Canada, [2]Carleton University, [3]Amphibia-Nature

Since the 1990s, occurrences of severely malformed amphibians have attracted scientific and public attention. However, the full extent and the causes of high rates of amphibian deformities continue to elude researchers. Understanding the spatial distribution of amphibians with developmental abnormalities is essential to help identify potential causes of the malformations and to determine the usefulness of this measure as an indicator of the state of the local environment. We analyzed data from 36,797 anurans (frogs and toads) examined at sites in agricultural areas of southern Quebec, Canada, over an 18-year period. While there was variation in the abnormality frequency across anuran species, on average, 4.7% of all individuals observed exhibited limb or eye malformations, which is consistent with an expected 5% natural baseline abnormality frequency. When the observations were divided into collection events (30 animals of one species observed at one site in one year), there was pronounced variation among sites: although 84% of the study sites had frequencies of 5% or less, one study site had a 42% average abnormality prevalence. The highest abnormality frequency observed using the collection event approach was a 96.8% abnormality frequency (or 29 malformed individuals of the 30 animals observed in that collection event). The most commonly found abnormality was malformed limb, which accounted for 45% of total abnormalities. Conversely, only 6% of abnormalities consisted of polymelia, or multiple limb deformity. The geospatial analyses conducted to determine "hotspot" clusters of high-abnormality prevalence resulted in a significant hotspot cluster being identified. The epidemiological analyses conducted as a follow-up to the spatial analyses suggested that high rates of amphibian developmental abnormalities show patterns associated with land use. As deformity hotspot distribution appears to be correlated with local environmental factors, this suggests the possibility of obtaining information concerning the state of the local environment from an analysis of amphibian deformities.

Sublethal effects of neonicotinoid pesticides on wood frogs: Mesocosm exposures (PL)

Stacey Robinson[1], Stacey Lee-Jenkins[2], Sarah Richardson[1], Vance Trudeau[3] and Bruce Pauli[1]

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Neonicotinoids are being prophylactically used on a variety of principal field crops in Canada. There is a growing concern for the potential impacts of neonicotinoids on Canadian aquatic ecosystems because most of the neonicotinoid treatment is not taken up by the target plant, rather it persists in the environment, leaches into soil and enters surface waters through run-off events. Health Canada's Pest Management Regulatory Agency (PMRA) is currently re-evaluating imidacloprid, thiamethoxam and clothianidin for their potential risk to non-target organisms. Amphibians such as frogs are susceptible to potential toxic effects of neonicotinoids because they use agriculture-associated aquatic ecosystems for reproduction, development and growth, and therefore are chronically exposed during sensitive periods. In fact, the intensive use of pesticides on crops has been identified as a contributor to aquatic ecosystem degradation and population declines of amphibians. The duality of the life cycle of amphibian species and sensitivity to environmental stressors make them ideal indicators to monitor the effects of neonicotinoids on non-target organisms. The objective of our project was to characterize the sublethal effect(s) of exposure to ecologically-relevant levels of two neonicotinoid pesticides on a Canadian native amphibian species, wood frogs (*Lithobates sylvaticus*), during their aquatic life-cycle stage using outdoor mesocosms (replicating semi-natural conditions). We exposed tadpoles to solutions of two commercial neonicotinoid formulations with the active ingredients imidacloprid and thiamethoxam and assessed survival, growth, behaviour and leukocyte counts. There was a significant effect of treatment on mean survival at metamorphosis (F6,36=4.47, p=0.003) where the 10 and 100 $\mu g \cdot L^{-1}$ imidacloprid treatment groups had greater survival compared to 1 $\mu g \cdot L^{-1}$ imidacloprid and 1 and 10 $\mu g \cdot L^{-1}$ thiamethoxam treatment groups (post-hoc Tukey test, $p<0.05$). There was no significant effect of treatment on body size (or mass) or development time after controlling for mean survival ($p>0.05$). Up to three weeks after exposure to neonicotinoids there was some evidence that the anti-predator response behaviour was affected in juvenile wood frogs. Overall, sublethal effects were detected in wood frogs after chronic exposure to environmentally-relevant surface water concentrations of imidacloprid and thiamethoxam.

Forty-year decline of organic contaminants in eggs of herring gulls (*Larus argentatus*) from the Great Lakes, 1974 to 2013 (PL)

Shane de Solla[1], Chip Weseloh[1], Kimberley Hughes[2] and Dave Moore[1]
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In the early 1970s, herring gulls (*Larus argentatus*) and other fish-eating colonial waterbirds in the Canadian Great Lakes had elevated embryonic mortality and poor reproductive success. Early studies indicated that their poor health was related to elevated organic contaminants. Those studies soon led to the adoption by Environment Canada of the herring gull and its eggs as an indicator species of health and contaminant exposure for fish-eating wildlife in the Great Lakes; thus the Great Lakes Herring Gull Egg Monitoring Program (GLHGEMP) was born and continues to this day. Due to its fish-eating diet, its non-migratory behaviour on the Great Lakes, and the fact that it is well-studied and well-known to the public, the herring gull is an excellent indicator species for organic contaminants on the Great Lakes. Our objective was to report on current and historical concentrations as well as changes in rates of decline or stabilization, of legacy contaminants (polychlorinated biphenyls (PCBs) and organochlorine pesticides) in herring gull eggs for 40 years of monitoring, from 1974 to 2013. POP burdens in eggs of herring gulls have declined considerably at 15 colonies monitored from the 1970s or early 1980s to 2013. Concentrations of PCBs (Aroclor 1260:1254 1:1 equivalents), for example, ranged between 50.1 and 165.6 $\mu g{\cdot}g^{-1}$ during 1971 to 1974 among the 10 colonies monitored, whereas by 2013 they ranged between 2.84 and 14.8 $\mu g{\cdot}g^{-1}$. Nonetheless, the rates of decline in POPs in herring gull in the Great Lakes were generally lower in later years, and for many colonies, concentrations have stabilized in the last few years. Temporal trends in PCB congener profiles in eggs were examined by grouping PCB congeners according to structure-activity relationships. Significant temporal changes in the percentages of non-metabolizable congeners (i.e., increase) and congeners metabolized by P450 1A enzymes (i.e., decrease) were evident in eggs from at least some colonies. It is not clear if these distinct temporal patterns are related to changes in metabolism following changes in PCB burdens or changes in bioavailability of congeners in the environment. Despite bans in production, the persistent nature of these legacy organic contaminants means that they continue to be available and accumulate in biota.

Identifying effects of wintering area on contaminants in White-winged Scoters: An isotopic approach (PL)

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Events during one stage of the annual life cycle of migratory birds can have lasting effects (i.e., carry-over effects) that influence demographic parameters in subsequent seasons. We studied migratory connectivity and potential carry-over effects in a declining population of sea ducks. We measured stable isotope values of carbon,

$\delta^{13}C$, and nitrogen, $\delta^{15}N$, in head feathers to assign breeding white-winged scoters (*Melanitta fusca*; hereafter scoters) to either Atlantic or Pacific winter populations. The discriminant function for $\delta^{13}C$ and $\delta^{15}N$ correctly classified 93% of scoters sampled from these two winter areas. We then applied this classification scheme to head feathers of females breeding at Redberry Lake, Saskatchewan, and Cardinal Lake, Northwest Territories, to stratify each breeding population by winter provenance. We evaluated carry-over effects associated with winter location of females breeding in Saskatchewan by testing for differences in (i) nesting phenology, (ii) clutch size, (iii) mid-incubation body mass, (iv) nest success, and (v) concentrations of trace element contaminants (cadmium (Cd), mercury (Hg), selenium (Se) and lead (Pb)) in blood, between strata of putative winter origin. Breeding females from the Atlantic coast had later dates of nest initiation, greater mid-incubation body mass, and also had higher concentrations of Cd (one year only), Pb, and Se, relative to birds from the Pacific. Neither nest initiation date nor mid-incubation body mass, however, were related to contaminant concentrations in blood. We found no differences in clutch size or nest success between putative winter strata. Our study detected carry-over effects in the Saskatchewan population that merit further attention.

Methylmercury metabolism across six Arctic marine mammals: Interspecies differences in exposure and neurotoxic risk (PL)

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Arctic marine mammals bioaccumulate some of the highest methylmercury burdens of any species, though little is known about internal metabolic processes and ultimately adverse outcomes and inter-species sensitivities. Here we performed a comparative study whereby liver and brain tissue was studied from polar bears (*Ursus maritimus*), pilot whales (*Globicephala* spp.), harbour porpoises (*Phocoena phocoena*), narwhal (*Monodon monoceros*), Baikal seal (*Pusa sibirica*), and ringed seal (*Pusa hispida*). For most animals, the brain was dissected into several regions of functional significance (frontal cortex, temporal cortex, occipital cortex, basal ganglia, brain stem, cerebellum, hippocampus, hypothalamus, thalamus, and pituitary). In all tissues, total and organic (methyl) mercury were determined. In some cases selenium (which binds to mercury in a 1:1 molar ratio) was measured, in addition to other persistent contaminants (e.g., polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs)). To explore whether exposures were of neurological concern, a suite of neurochemical biomarkers (e.g., NMDA receptor) was measured across the brain regions and then related back to the measured contaminants. Several results are noteworthy and highlight tremendous inter-species variability in exposure and risk. Across species, the highest mercury levels were generally found in the pituitary tissue. While in most brain regions organic mercury was more than 80% of total mercury, in the pituitary this value was only ~50%. Brain mercury content was much greater in pilot whales, narwhal, and harbour porpoises (many exceeded 10 ppm) versus polar bear, ringed seal, and

Baikal seal (few exceeded 1 ppm). Changes in some neurochemical biomarkers could be related to brain mercury levels in the aforementioned species with the highest mercury exposures. In a sub-study of polar bears, PCBs and PBDEs did not relate to any neurochemical biomarker though PFCs did. Differences in selenium handling and mercury demethylation are also apparent across species. Overall, these results suggest that certain marine mammals (pilot whales, harbour porpoise) accumulate mercury at levels capable of causing subclinical neurological effect, but others (e.g., polar bears) may have developed unique schemes to sequester or eliminate mercury and thus limit brain exposures.

S.W.O.T. analysis of the search for sentinels of exposure to air pollution (PL)

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The health effects of outdoor air pollution on urban populations continue to be of increasing medical epidemiological investigation in Canada and globally. With compelling evidence of negative health effects in people, identification of sensitive sentinel wildlife populations would be valuable. Sentinel species may be used to bridge the gap between exposure and disease associations generated by epidemiological studies, and to establish causality, which is the ultimate question. Working with wildlife populations serves the dual purpose of providing support for current human epidemiological studies and investigating the effects of chronic ambient exposure on animals. Benefits (strengths) of animal-based studies include: lifetime and multigenerational exposure studies, collection of multiple and serial samples, and procedures of varying invasiveness to strengthen weight of evidence, confinement of subjects to restricted areas or enclosures to control exposure, evaluation of effects at multiple tiers in the food web, and the option of complementary experimental studies with the same or similar species to gain insights into mechanisms of action. However, when it comes to identifying health effects of urban air pollution, understanding and quantifying exposure becomes a monumental task in itself, requiring multidisciplinary and creative techniques. Additionally, numerous potential confounding factors are intricately associated with observational and cross-sectional study designs, making identification of the real cause(s) of any biological changes challenging. We will discuss previous air pollution research using sentinel species and critically analyze our current study using a strengths, weaknesses, opportunities and threats (SWOT) analysis. We will identify methods of converting weaknesses and threats into strengths and opportunities, optimising chances of success in explaining the effects of ambient air pollution in Calgary on its European starling population.

Investigating whether industrial pollutant exposure affects the pre-migratory fuelling ability and the migration performance of two shorebird species (PL)

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Migration is an energetically costly process. Most birds must rely exclusively on endogenously stored energy to fuel prolonged bouts of migratory flight. Proper fuelling is important because it increases a bird's probability of surviving migration. Similarly, fat deposition rates are positively correlated with migratory speed, and early arrival at breeding grounds in the spring increases reproductive performance. Shorebirds exhibit some of the most extreme examples of pre-migratory fuelling, with many capable of more than doubling their mass prior to departure. Currently, however, many shorebirds are failing to sufficiently put on fat prior to departure from certain stopover locations. Oil pollution and legacy compounds, such as polychlorinated biphenyls (PCBs) and dioxins, have the potential to interfere with pre-migratory fuelling, and environmental contamination by industrial pollution has been identified as an important factor contributing to shorebird declines. However, the link between industrial pollutant exposure, impaired pre-migratory fuelling, and reduced migratory success have yet to be established in these birds. Our objective is to relate contaminant exposure to fuelling levels and stopover durations in two species of long-distance migratory shorebirds, sanderling (*Calidris alba*) and red knots (*Calidris canutus*). Birds were collected at two stopover locations along the Central Flyway: Chaplin Lake, Saskatchewan, a relatively uncontaminated site, and Corpus Christi, Texas, which is subject to contamination by oil spills and urban pollution. Contaminant profiles at each site were determined from sediment samples. Each bird's body condition was determined using its fat score, mass, and body morphometric measurements, and the bird's fuelling status was determined using plasma metabolite levels. We also fitted 20 red knots and 65 sanderling with coded nanotags, which transmit a signal that can be detected using radio telemetry array technology, to track the arrival, departure, and stopover durations of these birds. We found that body condition is closely related to plasma triglyceride concentrations and that birds at the more contaminated stopover sites exhibit lower fuelling rates.

Selenium toxicity in wildlife: Looking beyond fish (PL)

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Selenium (Se) toxicity and dynamics in fish have been studied by environmental scientists for many decades. Although much work remains to be done, it is well known that selenium accumulates in the aquatic food web. At certain thresholds, typically egg/ovary-based, Se can cause deformities and edema in developing fish embryos. Selenium toxicity in other oviparous vertebrates, especially those living at least part time in the terrestrial environment, is not well understood. Few studies have examined selenium toxicity in amphibians and reptiles that may be exposed to dietary selenium through aquatic prey. Recent studies have shown that current selenium guidelines for fish may be protective of amphibians based on an EC_{10} developed for *Xenopus laevis*. However, these frogs are not native to Canadian ecosystems and additional work using native amphibian and reptile species is required to determine species differences in sensitivity to selenium. Mammals are considered less sensitive to selenium than oviparous vertebrates, but there is increasing interest in understanding how fish-eating mammals may accumulate Se and if any adverse effects are relevant. Finally, significant data gaps exist for selenium toxicity in juvenile oviparous vertebrates including fish, birds, amphibians, and reptiles. These data gaps make effects assessments for ecosystems exposed to selenium challenging to regulators and industry scientists. This presentation will identify these data gaps and present research attempting to address these new challenges in selenium research.

Ecology and opportunities to study ecotoxicology of woodland caribou, wolves, and black bears in the Saskatchewan Boreal Shield (PO)

Philip McLoughlin[1]
[1]University of Saskatchewan

In 2014, the University of Saskatchewan, together with partners in the mining industry and government, initiated a multi-faceted study aimed at reducing uncertainty surrounding the status of Threatened woodland caribou (*Rangifer tarandus*) inhabiting the Saskatchewan Boreal Shield. In the absence of data on population status, trend, biology, and critical habitat, communities and companies of the region are afforded little guidance as to how activities must be mitigated to allow for acceptable practices under the *Species at Risk Act*. Of particular concern are implications for developing access and infrastructure for northern communities and sustainably developing natural resources, especially as they pertain to the mining industry. Our primary goals are to understand how caribou habitat and populations respond to conditions of high natural but low anthropogenic disturbance (localized centres of human activity, including mining), and how we can define critical habitat by relating survival and reproduction of individual caribou to their unique experiences of the environment (including habitat, fire, and exposure to predation and competition). Our five-year research plan is founded in basic and applied ecology to study woodland caribou habitat in northern Saskatchewan, the population and behavioural ecology of caribou, and that of their natural predators (wolves (*Canis lupus*) and black bears (*Ursus americanus*)). As part of this work, we are collecting samples of captured caribou, wolves, and black bears in areas of resource extraction, and will be in a position to conduct a study of ecotoxicology. This poster will highlight our collaborative study and opportunities to engage additional researchers to help add expertise in ecotoxicological research to our program.

Relationship between body condition and contaminants in overwintering canvasbacks (*Aythya valisineria*) in the Lake St. Clair/St. Clair River Area (PO)

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Overwintering canvasbacks (*Aythya valisineria*) were collected in the Lake St. Clair/St. Clair River area in the winter of 2008-09 and 2010, and livers were analyzed for polychlorinated biphenyls (PCBs), organochlorine pesticides, brominated flame retardants, mercury (Hg) and selenium (Se). Hepatic concentrations of organochlorines, brominated flame retardants, and Hg in all birds were low and Hg concentrations were below levels associated with adverse effects on survival. Hepatic concentrations of Se were largely low but were more variable among birds, with 25% of canvasbacks showing concentrations considered elevated and 5% (i.e., one bird) with a concentration exceeding the threshold associated with toxicity. Significant increases in hepatic concentrations of Hg and Se, sum PCBs, p,p'-DDE and other organochlorines were evident over the four month period of collection in which mean concentrations in January and/or February were significantly higher than mean concentrations in November when overwintering birds arrived in the study area. The largest increases were most often between December and January which also coincided with the period when birds from Lake St. Clair moved, following freeze-up of the lake, to the St. Clair River to forage. Simultaneous with the increase in body burdens, body condition of the birds declined from December onward. Although it is unclear to what extent possible changes in diet, level of contamination and/or declines in lipid reserves following movement to the St. Clair River contributed to observed temporal trends in body burdens of overwintering canvasbacks, our evidence suggests that a reduction of body condition, through loss of fat reserves, may have contributed to the higher body burdens in winter in canvasbacks.

Bioremediation and Reclamation

Biogeochemical instability of oil sands composite tailings undergoing reclamation (PL)

Lesley Warren[1]
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Oil sands tailings are important, globally relevant sulfur (S) reservoirs, known to contain active and diverse microbial communities. As evidenced by increasing S emissions from the oil sands, active biogeochemical S cycling within composite tailings (CT; a mixture of tailings, post-processed sand and gypsum, used for dry reclamation) is likely; however, the S biogeochemistry of these residues has not been investigated to date. Drill samples were collected in December 2012 from five depths spanning 36 m in a CT deposit (Fort McMurray, Alberta, Canada) for geochemical and metagenomic analyses. Results establish widespread microbial S biogeochemical cycling within the CT deposit. Porewater H_2S was detected extensively throughout the deposit with background levels of 14-23 µM and a concentrated pocket of 300 µM occurring at depth. Porewater Fe^{2+} (1-40 µM) was detected only within surficial CT depth samples. Current observed Fe^{2+} concentrations are not sufficient to sequester the levels of H_2S generated by CT, indicating untreated CT deposits may become a net source of S emissions over the longer term, as generated H_2S at depth migrates to the surface. Metagenomic (Illumina) characterization revealed highly diverse CT microbial communities, with 21 different phyla encountered overall and one-third of these presenting as candidate divisions. The cultivation-independent identification of several known iron-reducing bacteria (IRB) and sulfate-reducing bacteria (SRB) within these communities was consistent with observed positive growth in IRB and SRB functional metabolic enrichments. Furthermore, two depth-dependent structurally distinct communities emerged: a surficial CT zone of Fe^{3+} reduction and an underlying zone of SO_4^{2-} reduction, from multivariate statistical analyses of phylogenetic data (UniFrac). The emergence of a distinct, surficially constrained IRB zone, despite ~65% of the total bacterial community putatively having the capacity for Fe^{3+} reduction over the entire deposit depth, and evident and increasing Fe^{3+} sources down core, suggests IRB limitation by some other factor than their TEA substrate. Indeed UniFrac analyses identified that the differentiation in microbial communities occurring in these Fe and S zones was driven by environmental parameters of DOC, ORP and salinity; revealing that IRB may be unable to access the more complex residual OC constituents of these oil sands waste materials (i.e., bitumen, naphtha). Pilot reclamation for CT is currently focusing on capping CT with a freshwater fen, which may provide a more labile OC source for CT associated IRB, potentially stimulating greater H_2S sequestration through FeS formation. These processes will be evaluated in the on-going assessment of S biogeochemistry within untreated and treated CT as pilot reclamation proceeds.

Tracking microbial biomarkers in contaminated environments and bioremediation (PL)

Christopher Weisener[1]
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In many cases the activity of microorganism will directly impact the chemical conditions in both surface and subsurface water column environments controlling the fate of nutrients and contaminants alike. However, we face several daunting challenges when defining the bioremediation strategy of choice. Often the application is not straightforward since in many cases the anthropogenic disturbance has altered the local landscape, creating problems when defining the remedial end goal. Questions arise such as what are the baselines or reference systems that can be used and hopefully applied to support a remedial action of choice? What indices can be used to study the long-term and short-term controls on the mobility, cycling, and bioavailability of toxic metals and organic contaminants? In many cases the balance of chemical oxidizing and reducing components in water will control the development of chemical gradients we observe in either natural and/or applied systems (e.g., constructed wetlands or bioreactors). In some instances the biogeochemical system itself will determine the direction and onset of specific metabolic pathways as defined by their favourable thermodynamic outcome for most bioremediators (i.e., microorganisms). Often the degree of chemical alteration (toxicity or degradation products) can be directly linked to the proportion of their biological activity. Over the course of this century it will be important to identify cost effective/low maintenance solutions for treating contaminants in Canada's watersheds. Understanding the role of microbial drivers will be crucial for the optimization of a chosen bioremediation strategy. Often we have limited understanding of the role of good bacteria (facilitators) vs. bad bacteria (poisoners) in applied technologies, which remain a black box from the perspective of optimum growth and treatment conditions. From a temporal and spatial perspective, tracking the taxonomic and functional diversity in different settings (e.g., historically impacted vs. none impacted sites) could allow a better understanding of how applied systems should function. In this presentation, applied case studies investigating wetland function and the application of bioreactors will be discussed, which focus on linking physicochemical processes to microbial community function using next generation metagenomic and metatranscriptomic tools. This approach can be used to ultimately define and characterize baseline systems and/or optimize or monitor a chosen applied technology from a failure or functional aspect.

In situ bioremediation of saturated mine materials (PL)

James Harrington[1]
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Biological treatment systems can offer relatively low cost and minimal maintenance closure options for metal-rich mining waters. Bioremediation applied *in situ* can efficiently remove metals from solution, precipitating metals and metalloids as

insoluble metal sulfides or as reduced elemental forms or metal oxides. Assuring the stability of the precipitated metals is a key challenge for metals bioremediation. The biogeochemical processes that provide stability of precipitated metals in saturated systems focus around the formation of excess iron sulfide (FeS) which act as a redox buffer that controls the potential for metals resolubilization. Natural analogues of ore bodies such as roll-front uranium deposits show that FeS can limit metals solubility in natural systems. Formation of excess FeS as a second step after the initial bioremediation also provides residual treatment capacity when bioremediation reagents have been exhausted. This approach allows for infrequent maintenance or treatment cycles that allow more passive water treatment.

What is the fate of a lubricating oil in Canadian soils? (PL)

Amy Gainer[1] and Steven Siciliano[1]
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In the U.S., approximately 32% of used lubricating oils are released to the environment. Some pipeline equipment in Canada sprays lubricating oil during operations and releases a small amount to the soil. Soils have the ability to degrade parts of lubricating oils; however, after a 25-year lifespan lubricating oil can accumulate in the soil. We are interested in determining the fate and toxicity of a specific lubricating oil in Canadian soils. Soils below certain pipeline equipment with engines may contain fresh, used, and aged lubricating oil. Fresh lubricating oils are mainly mineral oils in the carbon range of C9 to C30 with lesser amounts of chemical additives like phenols and zinc salts. Used lubricating oils are similar to fresh lubricating oils but may contain additional compounds like polycyclic aromatic hydrocarbons. Aged is a descriptive term for lubricating oil found in the soils below the pipeline facilities exposed to fresh and used lubricating oils that are at varying stages of degradation. Aged lubricating oil in soil has varying amounts of organics and inorganics. We are interested in the processes influencing lubricating oil degradation in soils across Canada: we hypothesize that most of the lubricating oil degrades and that degradation will vary depending on regions in Canada and age of lubricating oil. To determine the factors limiting or driving degradation of lubricating oils in Canadian soils, chemical and biological indicators of degradation were applied. The research is part of the exposure assessment for the Human and Ecological Risk Assessment of the lubricating oil. The outcomes from the research contribute to improved management of industrial sites exposed to lubricating oils and the use of environmentally friendly engine oils.

Treatment of contaminated waters using a phased process-driven design of Constructed Wetland Treatment Systems (PL)

Monique Haakensen[1] and Vanessa Pittet[1]
[1]Contango Strategies

Constructed Wetland Treatment Systems (CWTSs) are biological systems that can be effectively used to decrease toxicity of contaminated waters to the environment. CWTSs can treat various contaminants in water when designed and executed in a scientifically guided manner. Based on a combination of biochemical, physical, and biogeochemical processes, they can be developed in a process-driven manner to effectively transfer, transform, degrade or remineralize contaminants as targeted by a specific design. While a site-specific approach is necessary to ensure success, there are several fundamental principles that can be applied to improve predictability and robustness. This presentation will use case studies to cover the principals of 1) site assessment and information gathering, 2) technology selection and conceptual design, 3) pilot-scale testing and optimization (controlled environment), 4) on-site demonstration-scale confirmation and optimization. Special emphasis will be given to the treatment of selenium and the fate and distribution of elements in these systems.

Variation in invertebrate community composition in a constructed wetland along environmental gradients (PO)

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Wetlands are areas of land whose soils are saturated with water for at least part of the year. Wetland classes (bogs, marshes, shallow water, swamps, and fens) are determined by fluctuations in water level, nutrient characteristics, water source, and the specific plant communities that are able to tolerate the particular hygric conditions. Wetland community composition and diversity are further constrained by key environmental gradients, reflecting spatial variation in salinity, moisture, and organic content of the soil. Fens, the most common wetland type in boreal Alberta, are susceptible to degradation or loss as a consequence of oil sands mine development. The 52-hectare Sandhill Fen on Syncrude Canada's lease is the largest full-scale fen to be built in the post-mining landscape. We are assessing how patterns of invertebrate community succession are influenced by environmental gradients within the Sandhill fen. The constructed uplands of the fen result in fine-scale variation in soil salinity and moisture content. This variation allows for drainage of surface water, causing a progressive increase in soil moisture and salinity as one proceeds from upland areas of the fen to lower elevations. Additionally, the substrate of the fen varies along elevations, with upland areas composed mainly of sand, and the lower portion increasingly dominated by peat. Each substrate type supports a different assemblage of vegetation. Pilot data were collected in summer 2014 to assess invertebrate biodiversity. Vacuum sampling was used to sample plant and soil-associated invertebrates, sticky traps to assess aerial insects, and pitfall traps to collect mobile, soil-dwelling invertebrates. Preliminary results indicate that upland areas support greater plant species diversity (richness; primarily planted trees and shrubs) than do wetter, lower areas, which were dominated by cattails and sedges. Cicadellidae (planthoppers), Thripidae (thrips) and Acari (mites) were the major groups of vegetation-associated invertebrates collected by vacuum sampling. Chironomidae (non-biting midges), Thripidae, Ceratopogonidae (biting midges), Cicadellidae, and Acari dominated collections from sticky traps placed in the upland and water-saturated zones. Mites were present in most of the soil suction samples, and in 10 of 11 pitfall traps. Additionally, most pitfall traps contained a species of Teleasinae wasp (Platygastridae), and various species of Muscidae (flies) and Carabidae (ground beetles). Pitfall traps yielded the highest frequency and average abundance of arachnids (spiders) and formicids (ants) among all traps. Pitfall trap results confirmed that the Sandhill Fen is in early invertebrate successional stages, as is typical for any wetland its age. We anticipate that variation in invertebrate community composition will reflect differences in plant species diversity along moisture and salinity gradients, but will be greater than similar locales, mature reference fens.

Applications of microbial profiling for optimized bioremediation strategies (PO)

Vanessa Pittet[1] and Monique Haakensen[1]
[1]Contango Strategies

It is known that natural microbial communities play a vital role in ecosystem health and bioremediation processes; however, fewer than 1% of microbes can readily be grown in a laboratory setting. While growth-based studies can provide essential information about metabolic capabilities of microbial communities, they do not reflect the composition and distribution of these assemblages in their natural setting. This poster will demonstrate how microbial community profiling can aid in the development and optimization of bioremediation processes. Commonly referred to as "microbiome" analysis or "metagenomics", these genetic tools provide understanding of the identities, abundance, and distribution of microbes in nearly any type of sample without requiring laboratory growth, and can be tailored to the detection of algae, archaea, bacteria, cyanobacteria, and fungi/yeasts. This allows for beneficial microbes to be identified, and bioremediation strategies adapted to provide the conditions that encourage desirable microbes to flourish in abundance and activity. These microbial community profiles can be correlated to "metadata" such as growth-based assays, geochemical or physicochemical data, or information about the health or remediation success of a specific site in order to optimize remediation efforts. Furthermore, what used to take months and thousands of dollars can now be accomplished in weeks for a tenth of the cost, opening up this exciting analytical tool for remediation project applications. This poster will present the current state of technology in genetic profiling of microbial communities and provide examples of output formats and applications.

Derivation and evaluation of adverse outcome pathways for the effects of cyclooxygenase inhibitors on reproductive processes in fish (PL)

Dalma Martinovic-Weigelt[1], Alvine Mehinto[2], Gerald Ankley[3], Jason Berninger[3], Timothy Colette[3], John Davis[3], Nancy Denslow[2], Elizabeth Durhan[3], Evan Eid[3], Drew Ekman[3], Kathleen Jensen[3], Michael Kahl[3] and Daniel Villeneuve[1]
[1]University of St. Thomas, [2]University of Florida, [3]U.S. Environmental Protection Agency

Cyclooxygenase (COX) inhibition is of concern in fish because COX inhibitors (e.g., ibuprofen) are ubiquitous in aquatic systems/fish tissues, and can disrupt synthesis of prostaglandins that modulate a variety of essential biological functions (e.g., reproduction). This study utilized newly generated high content (transcriptomic and metabolomic) empirical data in combination with existing high throughput (ACToR, epa.gov) toxicity data to facilitate development of adverse outcome pathways (AOPs) for molecular initiating event (MIE) of COX inhibition. We examined effects of a waterborne, 96-hour exposure to three COX inhibitors (indomethacin (IN; 100 $\mu g \cdot L^{-1}$), ibuprofen (IB; 200 $\mu g \cdot L^{-1}$) and celecoxib (CX; 20 $\mu g \cdot L^{-1}$) on the liver metabolome and ovarian gene expression (using oligonucleotide microarray 4 x15K platform) in sexually mature fathead minnows (n=8). Differentially expressed genes were identified (t-test, p<0.01), and functional analyses performed to determine enriched Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways (p<0.05). Principal component analysis indicated that liver metabolomics profiles of IN, IB and CX were not significantly different from control or one another. When compared to control, exposure to IB and CX resulted in differential expression of comparable numbers of genes (IB=433, CX=545). In contrast, 2558 genes were deferentially expressed in IN-treated fish. KEGG pathway analyses show that IN had extensive effects on oocyte meiosis and muscle contraction processes, which is consistent with physiological roles of prostaglandins in the fish ovary. Transcriptomic data was congruent with apical endpoint data—IN caused significant suppression in plasma prostaglandin F2 alpha concentrations, and reduced ovarian COX activity, whereas IB and CX did not. Ovulation status, chemical treatment, and their interaction did not have statistically significant effect on circulating vitellogenin. These findings were used to develop AOPs for COX inhibition (MIE) leading to reproductive failure (adverse outcome) via two series of key events—one associated with inhibition of reproductive behaviour, and another with the inhibition of oocyte maturation/ovulation. The contents of this abstract neither constitute, nor necessarily reflect, official U.S. Environmental Protection Agency policy.

The stoichiometric pathway analysis of steroidogenesis in fathead minnows (*Pimephales promelas*) (PL)

David Hala[1]
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The assessment of an organism's robustness or susceptibility is inherently dependent on a quantitative description of underlying metabolic network structure and function. Towards this end, the structural and functional analyses of metabolic networks are unified by stoichiometric matrix representations of metabolite mass balances (i.e., consumption and production) in interrelated enzyme-catalyzed reactions. In this presentation, I will present some of my work with applying the computational framework of constraints-based flux balance analysis (FBA) to assess effects of exposure to a synthetic estrogen, 17α-ethynylestradiol (or EE2), on male and female fathead minnow (*Pimephales promelas*) steroidogenesis and gonad growth. Specifically, a previously developed stoichiometric model of piscine steroidogenesis was augmented with a pseudo "Biomass Reaction" constraining tissue levels of various steroid hormones (as $\text{fmol}\cdot\text{mg}^{-1}$ gonad weight) to the generation of gonad growth. In addition, fold changes in expression of selected steroidogenic enzyme genes were also quantified and used to scale the minimum/maximum reaction bounds for selected reactions in the stoichiometric model. Simulations involved calculating optimal reaction flux distributions that "maximized" biomass generation (or gonad growth) and the identification of diagnostically altered reactions in EE2 exposed male and female fish. In our study, FBA prediction of steroidogenic effects and perturbed gonad growth in male and female fish agreed with *in vivo* experimentation. The approach presented may be an important step towards identifying disrupted steroidogenic pathways representative of compound-specific modes of action and allow incorporation of computational predictions with toxicological risk assessments.

Understanding the effects and mode of action of emerging contaminants (benzotriazoles) using RNA-sequencing in *Daphnia magna* (PL)

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Benzotriazoles (BTRs) are high production volume chemicals used as anticorrosive and antifreeze agents in a broad range of industrial applications as well as in households. Their high polarity, water solubility, and resistance to biodegradation have resulted in their ubiquitous presence and detection in surface waters, potentially impacting aquatic organisms. BTRs have therefore been listed as prioritized emerging contaminants under the Chemical Management Plan of the Canadian government in order to assess their ecotoxicological effects. The objective of this study was to measure sublethal chronic toxicity of 1H-benzotriazole (BTR), 5-methyl-1H-benzotriazole (5MeBTR) and 5-chloro-1H-benzotriazole (5ClBTR) using *Daphnia magna*, a renowned model for risk characterization of chemicals. A multi-level toxicological study combining genomic biomarker analyses and whole organism endpoints was used to identify impacted biological pathways and get insight into the molecular modes of action and impacts on population health. Results showed that the three compounds had similar acute toxicities (LC_{50} range from 20 to 60 $mg \cdot L^{-1}$) and that only 5MeBTR exhibited chronic effects at the organism level, with a significant increase of the number of molts after a 21-day exposure to sublethal concentrations. High-throughput RNA sequencing was used to identify differentially expressed genes and understand the mode of action of each of the three BTRs. A list of significantly over- and under-transcribed candidate genes (fold change = 2, $p<0.01$) will be further analyzed by qRT-PCR to confirm the observed transcriptomic response. Changes of gene expression will be linked to the protein level by measuring the corresponding protein activity when possible. Taken together, results of this study will give insight into the mode of action of emerging contaminants at different biological levels, providing an integrated method for environmental risk assessment.

Characterization of toxicity pathways of 2,3,7,8-TCDD, PCB 77, and benzo[a]pyrene in white sturgeon using whole transcriptome and proteome analyses (PL)

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Populations of sturgeons are considered threatened or endangered worldwide, and there is significant concern regarding the contribution of pollution to current declines in populations. However, risk assessment attempts are hampered by a lack of knowledge about the sensitivity of sturgeons to pollutants of concern. One class of pollutants of concern is dioxin-like compounds (DLCs), which are known to cause a variety of adverse outcomes in fishes. These outcomes can include craniofacial and cardiovascular malformations, pericardial and yolk sac edema, wasting syndrome, hepatotoxicity, reduced hemopoiesis, immune suppression, and impaired reproductive and endocrine processes. However, little is known about adverse outcomes and molecular mechanisms responsible for these effects in sturgeons. Therefore, this study aimed to predict possible adverse outcomes as a result of exposure to DLCs in sturgeons through identifying molecular mechanisms in white sturgeon (*Acipenser transmontanus*). Sturgeon were exposed to equipotent doses of two DLCs, 2,3,7,8-tetrachloro-dibenzo-p-dioxin (TCDD) and 3,3',4,4'-tetrachlorobiphenyl (polychlorinated biphenyl (PCB) 77) and one polycyclic aromatic hydrocarbon (PAH), benzo[a]pyrene (BAP), administered by intraperitoneal injection. Livers were excised and used for whole transcriptome and proteome analysis. Transcriptome analysis revealed 378, 493, and 529 genes were up-regulated by 2-fold or greater following exposure to TCDD, PCB 77, or BAP, respectively, while 296, 325, and 394 genes were down-regulated by 2-fold or greater, respectively. BAP altered both a greater number of genes and a greater diversity of genes relative to TCDD and PCB. TCDD and PCB shared several pathways known to be perturbed by exposure to DLCs in other fishes, such as genes involved in angiogenesis, heart and skeletal system development, sensory organ development and neurogenesis, metabolic pathways, apoptosis, and response to hormone stimuli. BAP perturbed several AhR-mediated pathways in common with TCDD and PCB 77, but also altered genes involved in muscle development, immune response, hemopoiesis, blood coagulation, and catabolism. This is indicative of BAP triggering both AhR-mediated and non-AhR mediated pathways while most effects of TCDD and PCB 77 were believed to be AhR-mediated. Proteome analysis will be used to link altered transcripts to transcribed proteins to identify similarities and differences in possible apical adverse outcomes between TCDD, PCB 77, and BAP in sturgeons.

Utilizing high throughput bioassays to characterize the bioactivity of complex environmental samples (PL)

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Bioassays can be employed to evaluate the integrated effects of complex mixtures of both known and unidentified contaminants present in environmental samples. However, such methods have typically focused on one or a few bioactivities despite the fact that the chemicals in a mixture may exhibit a wide range of activities. High throughput toxicology approaches that can rapidly screen samples for a broad diversity of biological activities offer a means to provide a more comprehensive characterization. To test this concept, twenty-four ambient water samples were collected, extracted, and screened for their ability to interact with or modulate over 80 different transcription factors using the Attagene subset of assays utilized by the U.S. Environmental Protection Agency's (USEPA's) ToxCast Program. Samples evaluated included 10 water samples collected in varying proximity to a wastewater discharge within the St. Louis River Area of Concern (AOC), MN; water collected at five sites along a spatial gradient centered on a wastewater discharge into the Maumee River, Ohio, U.S.; and eight samples collected in association with a nation-wide US Geological Survey Mixture Study. For samples collected along the St. Louis River AOC, the greatest number of biological activities was observed at locations closest to wastewater discharge, with as many as 13 endpoints responding. The Maumee River showed a gradient response in the number of observed activities, ranging from three positive assay responses observed far upstream of discharge to seven positive responses in water from the mixing zone. TGFb signaling and the aryl hydrocarbon receptor (AhR) activation were the biological activities observed at the lowest sample concentration (greatest dilution). For the U.S. Geological Survey mixture study sites, the number of positive responses ranged from 2 for a sample collected from the Iowa River, IA to 15 for a sample collected from the South Platte River, Colorado. Estrogen response elements and the AhR were activated at the lowest sample concentration. The biological activities identified for each site can now be mapped to established adverse outcome pathways to predict potential hazards to aquatic biota and/or selecting endpoints for subsequent site-specific effects monitoring. The contents of this abstract neither constitute, nor necessarily reflect, official USEPA policy.

The brominated flame retardant TBCO affects fecundity and transcript profiles of the HPGL-axis in Japanese medaka (PO)

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The novel brominated flame retardant, 1,2,5,6-tetrabromocyclooctane (TBCO) is an additive flame retardant which is marketed under the trade name Saytex BCL-48. TBCO has recently been investigated as a potential alternative to the major use brominated flame retardant, hexabromocyclododecane (HBCD), which could have major implications for significant increases in amounts of TBCO used. Yet there is a lack of information regarding potential toxicities of TBCO. Recently, results of *in vitro* experiments have demonstrated the potential of TBCO to modulate endocrine function through interaction with estrogen and androgen receptors and via alterations to the synthesis of 17-β-estradiol and testosterone. Further research is required to determine potential endocrine disrupting effects of TBCO *in vivo*. In this experiment a 21-day fecundity assay with Japanese medaka (*Oryzias latipes*) was conducted to examine endocrine disrupting effects of TBCO *in vivo*. Medaka were fed a diet containing either 607 or 58 µg $TBCO{\cdot}g^{-1}$ food, wet mass (wm). Fecundity, measured as cumulative deposition of eggs and fertilization of eggs, as well as abundances of transcripts of 34 genes along the hypothalamus-pituitary-gonadal-liver (HPGL) axis were measured as indicators of holistic endocrine disruption and to determine mechanisms of effects, respectively. Cumulative fecundity was 18% lesser by medaka exposed to 58 µg $TBCO{\cdot}g^{-1}$, wm food. However, fecundity of medaka exposed to 607 µg $TBCO{\cdot}g^{-1}$, wm food was not significantly different from that of controls. Organ-specific and dose-dependent alterations to abundances of transcripts were observed in male and female medaka. A pattern of down-regulation of expression of genes involved in steroidogenesis, metabolism of cholesterol, and regulatory feedback mechanisms was observed in gonads from male and female medaka which had been exposed to the greater concentration of TBCO. However, these effects on expression of genes were not manifested in effects on fertilization of eggs or fecundity. In livers from male and female medaka exposed to the lesser concentration of TBCO, greater expression of genes that respond to exposure to estrogens, including vitellogenin II, choriogenin H, and ERα, were observed. The results confirm the endocrine disrupting potential of TBCO and elucidate potential mechanisms of effects which include specific patterns of alterations to abundances of transcripts of genes in the gonad and liver of medaka.

Pesticides

Green eggs and atrazine (PL)

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As an embryo, the yellow-spotted salamander (*Ambystoma maculatum*) has a unique endosymbiotic relationship with the green alga, *Oophila amblystomatis*, that colonizes its egg capsule. This represents a plausible scenario whereby an herbicide might impair an amphibian, albeit indirectly. In this case, salamanders might be impacted via potential effects on the alga, at environmentally realistic exposures and durations. We set out to characterize the sensitivity of this relationship, measuring the sensitivity of the alga in isolation, but also within the egg mass, along with the developing salamander larva. We first needed to isolate and develop culturing methods for the alga so that near-standard toxicity testing protocols could be employed. Once validated, we found that *O. amblystomatis* under laboratory conditions was less sensitive than most standard species, with 96-hour EC_{50}s>100 $\mu g \cdot L^{-1}$, which are significantly greater than concentrations typically measured in the environment. As well, complete recovery from detected effects was rapid once atrazine was removed from test media. Next, we characterized the response of the alga and larval salamanders together in the egg mass under laboratory conditions. In this more realistic experiment, we again observed little to no impairment of the alga (or the embryonic salamanders) at 2-month exposures at more than 300 $\mu g \cdot L^{-1}$, again well above environmentally realistic concentrations. From this work, we conclude that atrazine poses no significant risk of toxicity to these green eggs or the salamanders therein.

Acute effects of imidacloprid and monocrotophos on migratory behaviour in white-throated (*Zonotrichia albicollis*) and white-crowned sparrows (*Zonotrichia leucophrys*) (PL)

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Current use agricultural pesticides have been shown to have caused neurological intoxication in both avian and mammalian animal models. Little is known about the direct impacts of pesticides on migratory behaviour and survival during migration of wild species. Avian exposure to pesticides prior to and during migration could cause changes in cognitive function and orientation behaviour, which are fundamental components of most birds' successful migration and are essential for survival and safe arrival at the breeding or wintering grounds. We evaluated how a short-term acute exposure to two insecticides, imidacloprid (neonicotinoid) and monocrotophos (organophosphate), each

with different modes of action, could cause physiological and behavioural effects in two migrant songbird species, the white-throated sparrow (*Zonotrichia albicollis*) and the white-crowned sparrow (*Z. leucophrys*) during a spring migration. As part of a pilot study, 25 birds (white-throated sparrow: n=9; white-crowned sparrow: n=16) were captured and taken into captivity and dosed once daily by oral gavage for 72 hours with 0 (water vehicle only), 12.5 $\mu g \cdot g^{-1}$ imidacloprid, or 0.40 $\mu g \cdot g^{-1}$ monocrotophos. Serial blood samples were taken to evaluate plasma cholinesterase and body mass was measured daily. Nocturnal Emlen funnel trials were used to determine the effects of a short-term sublethal exposure to insecticides on migratory activity, orientation, and recovery. Overall, there was no significant effect of oral dosing on survivability (X^2=0.87, df=2, p=0.80) and preliminary data shows that prior to insecticide exposure, birds were exhibiting a normal northerly directional preference. Data on effects during and post-dosing are currently being analyzed. Since migration is a critical event for many birds, this may affect the survival of both individual birds and their ability to arrive at the breeding grounds. Given the pronounced declines in many songbird species, there is an urgent need to assess whether extensive use of agricultural pesticides across the migratory range is capable of affecting bird migration.

Teratogenic and behavioural effects of imidacloprid on honey bees (*Apis mellifera*) (PL)

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Elevated winter mortality has been documented in North American honey bee (*Apis mellifera*) populations since 2006. The neonicotinoid imidacloprid has (published) teratogenic effects in chickens and documented negative effects on grooming behaviour in termites and white grubs. The purpose of this study was to assess palatability of neonicotinoids to honey bees, as well as to investigate teratogenicity and effects on grooming and hygienic behaviour in honey bees exposed to imidacloprid. An initial field palatability trial of nine hives exposed to three different neonicotinoids (clothianidin, thiamethoxam, and imidacloprid at 0,1,10 and 100 $\mu g \cdot L^{-1}$ each) demonstrated no significant difference in consumption among different concentrations of each neonicotinoid, in contrast to published laboratory-based palatability trials in honey bees. At the termination of the palatability trial, the hives exposed to imidacloprid were significantly weaker compared to the hives exposed to clothianidin and thiamethoxam. Assessment of teratogenic and behavioural effects of imidacloprid was performed using four groups of six hives fed one of four concentrations of imidacloprid in 1:1 sugar solution (0,1,10 and 100 $\mu g \cdot L^{-1}$). Teratogenic effects of imidacloprid were assessed by light microscopy of histologic sections of larvae and pupae sampled at regular intervals during embryogenesis. Grooming efficacy of Varroa destructor mites by honey bees was measured by the Varroa drop method, while hygienic behaviour was quantified by the freeze-killed brood assay. Analysis and comparison of teratogenic effects, grooming efficacy, and hygienic behaviour among experimental groups is in progress. The results

of this study are anticipated to contribute to understanding of the role of neonicotinoids in winter mortality of honey bees.

Assessing the acute and chronic toxicity of neonicotinoid insecticides to non-target aquatic species (PL)

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Neonicotinoids are the most widely used insecticides in the world. They are preferentially toxic to insects while displaying a low toxicity toward vertebrates; this selective toxicity has led to their rapid and ubiquitous use. However, neonicotinoids may negatively affect aquatic ecosystems because they are environmentally persistent and highly water-soluble, and thus are prone to leaching from the soil into surface waters via run-off events. Although non-target aquatic organisms may be adversely affected by these compounds, little information is available regarding the effects of neonicotinoids on aquatic invertebrates. The objective of this study was to assess the acute and chronic toxicity of four neonicotinoids (imidacloprid, thiamethoxam, acetamiprid, and clothianidin) to three species of freshwater invertebrates: *Lampsilis fasciola* (mussel), *Hyalella azteca* (amphipod), and *Hexagenia* spp. (mayfly). Neonicotinoid exposures were conducted in water-only systems, and test durations were 48 hours (mussel glochidia), 96 hours (mayfly), and 28 days (amphipod). Endpoints assessed included viability (ability to close valves) in mussel tests, survival and behaviour (number of animals inhabiting artificial burrows) in mayfly tests, and survival and growth in amphipod tests. Preliminary results revealed that amphipods were the most sensitive species to neonicotinoids, with 28-day LC_{50}s of 3, 4, 70, and 200 $\mu g \cdot L^{-1}$ for clothianidin, acetamiprid, imidacloprid, and thiamethoxam, respectively. Mussels were the least sensitive species: no effects on viability were observed following exposure to any of the four neonicotinoids at up to 1000 $\mu g \cdot L^{-1}$ for imidacloprid and thiamethoxam, and 500 $\mu g \cdot L^{-1}$ for acetamiprid and clothianidin. Effects of neonicotinoids on mayflies were variable. The 96-hour LC_{50} for imidacloprid was 400 $\mu g \cdot L^{-1}$, survival was 83% and 70% at the highest test concentration (100 $\mu g \cdot L^{-1}$) for acetamiprid and clothianidin, respectively, and no effects on survival were observed for thiamethoxam at concentrations up to 1000 $\mu g \cdot L^{-1}$. However, mayfly behaviour was affected at lower concentrations than survival for three neonicotinoids: more than 70% of animals were found outside of their burrows at concentrations of 10 $\mu g \cdot L^{-1}$ and higher for imidacloprid and acetamiprid, and at 1000 $\mu g \cdot L^{-1}$ for thiamethoxam. The maximum environmental concentration of neonicotinoids measured in 2012-2014 surveys of 16 stream sites in southern Ontario was imidacloprid at 10 $\mu g \cdot L^{-1}$, indicating that environmental concentrations may adversely affect sensitive aquatic species.

Impacts of neonicotinoid insecticides on aquatic insect emergence in prairie wetlands using *in situ* limnocorrrals (PL)

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Ecologically important wetlands adjacent to agricultural fields planted with neonicotinoid treated seed are susceptible to chronic neonicotinoid exposure from run-off and the long environmental persistence of these compounds. However, little information is available on the chronic effects of the different neonicotinoids used on a suite of economically viable crops to wetland invertebrate taxa. Field mesocosm experiments with neonicotinoids applied at environmentally relevant, chronic exposure concentrations can provide information to help interpret the effects of large-scale field applications. Here, *in situ*, multi-species wetland limnocorrals were used to compare the toxicity of different neonicotinoid active ingredients—imidacloprid (IMI), clothianidin (CLO), and thiamethoxam (THX)—to natural aquatic invertebrate communities. Over a 14-week study, the 21 limnocorrals were dosed weekly with one of the three neonicotinoids at rates of either 0.05 or 0.5 $\mu g \cdot L^{-1}$ for 8 weeks to achieve a chronic, repeated exposure scenario, followed by a 6-week recovery period. Water concentrations and aquatic insect emergence were monitored throughout the study and revealed decreasing emergence in the insect families Chironomidae (mid-summer) and Phryganeidae (early-autumn) in all treatments. Sixteen families and three chironomid tribes were identified over all treatments with two sensitive taxa exclusively emerging from the control units: *Callibaetis ferrugineus* (Ephemeroptera: Baetidae) and *Agraylea multipunctata* (Trichoptera: Hydroptilidae). Control limnocorrals harboured greater diversity of taxa, cumulative abundance, and emergent insect biomass than all treatments. These data compared favourably with data from single-species tests (*Chironomus dilutus*) conducted in the lab. The community assessment approach offered by the limnocorrals provides new insight on the effects of these insecticides to important wetland ecosystems and should inform regulations for setting appropriate aquatic life reference values for neonicotinoids.

Neonicotinoid insecticide mixtures: Characterizing cumulative toxicity under acute exposure scenarios (PL)

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Neonicotinoid insecticides are currently the most widely purchased and heavily used class of agrochemical pesticides world-wide. Commonly applied as seed-treatments or foliar sprays, these compounds are used to protect crops against a broad spectrum of biting-sucking insects. Neonicotinoids are highly water soluble, so following application these compounds are likely to move into nearby aquatic systems. Here they can display multi-season persistence, thus posing a risk to resident aquatic insect

populations. Due to application of different neonicotinoids within the same watershed or between growing seasons, neonicotinoids are frequently detected in aquatic systems as binary and tertiary mixtures. However, the cumulative toxic effects of these neonicotinoid mixtures are poorly understood. This research aims to fill that knowledge gap by characterizing the cumulative toxicity of binary and tertiary mixtures of select neonicotinoids (imidacloprid, clothianidin, and thiamethoxam) to the sensitive aquatic insect *Chironomus dilutus* under acute exposure scenarios. Preliminary assessments of single-compound toxicity in 96-hour laboratory tests have been completed, yielding toxicity threshold values (LC_{50}, 95% confidence intervals in parentheses) of 4.63 (3.96 - 5.41), 5.93 (5.29 - 6.65), and 55.34 (43.98 - 69.64) $\mu g \cdot L^{-1}$ for imidacloprid, clothianidin, and thiamethoxam, respectively. These values will be used to develop parametric models, which will be statistically compared to the toxicity of binary and tertiary mixtures from similar laboratory studies, using the MIXTOX approach. MIXTOX applies a regression-based experimental analysis, assessing the toxicity of mixtures at different dose-ratios and dose-levels (fixed-ray design), allowing for a comprehensive examination of cumulative toxicity. Results obtained through these experiments will shed light on the cumulative toxicity of neonicotinoid mixtures under acute exposure scenarios, allowing for an assessment of whether these compounds display concentration-additive toxicity and what risk these insecticide mixtures pose to aquatic ecosystems.

Sublethal effects of the neonicotinoid imidacloprid on developing sockeye salmon (*Oncorhynchus nerka*) (PO)

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Current-use pesticides are a concern for the health of anadromous salmonids throughout the Fraser River Basin in British Columbia, Canada. Neonicotinoids are a class of widely used insecticides that have been detected in ng-$\mu g \cdot L^{-1}$ concentrations in surface waters globally, including those in the Fraser River Basin, yet few studies have examined the potential sublethal effects of neonicotinoids on aquatic vertebrates. This study examined the effects of a neonicotinoid insecticide, imidacloprid, following either a single pulse or chronic exposure, on critical early life stages of a wild salmon species, sockeye salmon (*Oncorhynchus nerka*). In this study, 4 concentrations of imidacloprid (0.15, 1.5, 15 and 150 $\mu g \cdot L^{-1}$) plus a water control were tested in a single pulse exposure experiment during the fertilization process and in a chronic exposure experiment that was initiated immediately post-fertilization; both experiments continued through to the swim up fry developmental stage. Three unique offspring sets (crosses) were tested within each experiment and all treatments were conducted in duplicate. Imidacloprid did not affect survival, deformity rates or morphometrics in either pulse or chronic exposures, although differences between crosses within imidacloprid treatments were evident. Gene expression analyses to examine alterations in hepatic endocrine, nervous and immune system transcript abundance and hormone concentration analyses are ongoing to further investigate the sublethal effects of imidacloprid in early life stage sockeye salmon. Supported by Fisheries and Oceans Canada National Contaminants Advisory Group.

Persistence and toxicity of Garlon XRT® and Arsenal Powerline® in soils along transmission right-of-ways in the Yukon Territory (PO)

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Vegetation management along transmission right-of-ways (ROWs) in the Yukon consists of mechanical methods such as mowing and brushing. When mowed, fast growing woody species that dominate these ecosystems need to be controlled frequently. Thus other vegetation control measures, such as herbicide application, that can reduce management costs, improve long-term effectiveness, and reduce environmental risks are being examined. This research aims to determine the persistence and toxicity of Garlon XRT® (triclopyr) and Arsenal Powerline® (imazapyr) in soils along Yukon ROWs. Soil samples from field application (foliar spray, cut stump and point injection) are currently being analyzed; however, it is hypothesized that the herbicides will persist longer in northern soils when compared to average half-lives reported for temperate soils. This can be attributed to the length of time the soil is frozen, reducing attenuation rates through both microbial and chemical degradation. Invertebrate toxicity assays will be utilized to determine the ecological risk associated with application on five Yukon ROWs. It is hypothesized that less than 25% of the soil invertebrate population will be affected when applied at maximum application rates.

Metal Mixture Toxicity

Characterizing multiple metals (Ag, Cd, Cu, Ni, Pb and Zn) interactions on metal uptake in the freshwater snail, *Lymnaea stagnalis* (PL)

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Developing conceptual frameworks for the prediction of metal mixtures aquatic toxicity is a challenge currently addressed by metal ecotoxicologists. One proposed approach is to extend the biotic ligand model (BLM), a conceptual framework that has been extensively used to predict single metals bioavailability, to a multi-metal version. The BLM describes metal-organism interactions as a chemical equilibrium between the free metal ions Mz^+ in solution and sensitive biological sites (biotic ligands), with the free metal concentration $[Mz^+]$ being the best predictor of metal toxicity. Accordingly, the latter can be affected by the presence of other cations in solution (e.g., Ca^{2+}, Na^+ and H^+) via different ways: (i) direct competition for binding to the biotic ligands and (ii) competition for binding to aqueous ligands (e.g., Cl^-, OH^- and dissolved organic matter) influencing the metal speciation in solution (i.e., $[Mz^+]$). By assuming that these competition reactions could explain much of the metals interactions for toxicity, multi-metals BLMs have been proposed to explain particular toxicity datasets. Yet, a major recognized hurdle for the development of such models is the lack of mechanistic knowledge of how metals specifically affect each other's toxicity (e.g., which metals share common membrane transporters?). The present study aims to gain some insights into these mechanisms. We are investigating binary mixture effects on the uptake of six metals (Ag, Cd, Cu, Ni, Pb and Zn) in the freshwater snail, *Lymnaea stagnalis*. This species has been shown to be highly chronically sensitive to trace metals such as Pb or Ni (Brix *et al.* 2012 - Aquatic Toxicology 106 - 107, 147- 156 and Niyogi *et al.* 2014 - Aquatic Toxicology 150, 36-44), with ionoregulatory disturbance (e.g., Ca^{2+} uptake inhibition) a postulated mechanism for toxicity. Short-term metal uptake in *L. stagnalis* is measured for each metal alone and in combination with a second metal, using radio-isotopes (Ag-110m, Cu-64, Cd-109, Ni-63 and Zn-65) as tracers. Metal exposure concentrations are within the range of chronic toxicity (based on 14-day toxicity on growth). Results are analyzed using the BLM framework, allowing the determination of log K values for metal binding to membrane transporters, as well as the characterization of the type of metals interactions occurring at the site of uptake (competitive or non-competitive). Supported by National Sciences and Engineering Research Council CRD, IZA, ICA, CDA, NiPERA, Rio Tinto.

Effects of waterborne metals in binary mixtures on short-term gill metal-binding and ion uptake, and toxicity in rainbow trout (*Oncorhynchus mykiss*) (PL)

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The current Biotic Ligand Models (BLMs) do not account for the effects of metals in mixture, although organisms inhabiting the metal-contaminated natural waters are almost always exposed to multiple metals at elevated concentrations. Metals are known to share common uptake pathways in fish gill. For example, cadmium (Cd) and zinc (Zn) are both taken up by calcium transport pathway, whereas copper and silver are absorbed by sodium transport pathway. Thus, metals sharing a common uptake pathway should exhibit competitive interactions when they are in mixture, and metals with different uptake pathways should not interact in the gill. The BLM framework is based on the short-term gill metal-binding characteristics of the fish gill. The objective of the present study was to examine the interactive effects of metals (cadmium, copper, nickel, silver and zinc) in binary mixtures on short-term (3-hour) branchial uptake of metals and ions (calcium and sodium) in juvenile rainbow trout (*Oncorhynchus mykiss*) during acute waterborne exposures. We found that both copper and zinc reduced cadmium uptake and vice versa; however, nickel did not have any effect on cadmium uptake. Interestingly, silver was found to stimulate copper uptake, whereas copper had no effect on silver uptake. The inhibitory effect of cadmium and zinc in mixture on calcium uptake was greater than that of cadmium or zinc alone. Similar additive inhibition of calcium uptake was also recorded with cadmium and copper in mixture. The inhibitory effect of copper and silver in mixture on sodium uptake was greater than that of copper or silver alone. Cadmium did not affect sodium uptake, and no additive inhibition of sodium uptake was observed with cadmium and copper in mixture. Acute toxicity assessments conducted with cadmium and zinc in mixture demonstrated that zinc did not affect the toxicity of cadmium, but cadmium increased zinc toxicity in an additive manner. Overall, our findings indicate that gill-binding characteristics of metals in binary mixture do not always follow the predicted pattern; nevertheless toxicity can be reasonably explained by simple fractional response additions. These observations may have useful implications for adopting the BLM approach to assess metal-mixture toxicity in fish.

Considerations for assessing multi-metal toxicity in fish: Effects of lead and cadmium mixtures on gill function (PL)

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Until recently, relatively few studies had attempted to determine how metal mixtures adversely affected processes mediated by the fish gill, the initial target of waterborne metals and the site of gas exchange, acid-base and ion regulation. Two metals likely to be encountered together in contaminated waters are cadmium (Cd) and

lead (Pb), which may arise from mining and smelting operations, the burning of coal, and electronic wastes. To determine how rainbow trout (*Oncorhynchus mykiss*) respond to Cd plus Pb mixtures we exposed the fish to these metals in soft (Ca^{2+}<100 μmol·L^{-1}) slightly acidic (pH ~ 6) water, typical of many waters in the Canadian Shield. Previously, we reported that acute exposure (3h) of trout to Cd plus Pb mixtures had greater than additive inhibitory effects on both Ca^{2+} and Na^+ uptake, which could potentially exacerbate disturbances to ion balance and cause greater toxicity (Birceanu *et al.* 2008. Aquat. Toxicol. 89:221). To test the hypothesis that the physiological and toxicological effects of Cd plus Pb mixtures persisted over longer periods we exposed trout to relatively low, more environmentally relevant concentrations of these metals for 3-5d. Repetitive blood sampling experiments revealed that exposure to Pb alone (26 nmol·L^{-1}) was less toxic than Cd alone (6 nmol·L^{-1}). However, Cd plus Pb mixtures (7 nmol·L^{-1} and 45 nmol·L^{-1}) resulted in greater than additive mortality (80%) by 5 days. Exposure to either Cd or Pb alone for 3d inhibited Na^+ uptake, which was partially offset by decreases in diffusive Na^+ loss across the gill. There were no effects of Pb alone on plasma ion balance, but Cd plus Pb exacerbated Cd-induced reductions in plasma Ca^{2+}, and led to significant decreases in plasma Na^+ and Cl^-, and osmolality. No effects on Na^+/K^+ ATPase activity were noted. Nor were there disturbances to arterial blood pH or PO2, indicating that Cd plus Pb mixtures had no adverse effects on blood acid-base regulation or gas exchange. Attempts to predict how these mixtures interacted at the gill to cause toxicity using the concentration addition (dose addition) model were unsuccessful in this and our previous studies (Winter *et al.* 2012. Ecotoxicol. Environ. Safety 83:16). This model uses the Toxic Unit (TU) approach to predict toxicity, in which 1TU is defined as the metal concentration required to kill 50% of fish over a defined period. However, the model requires that the constituent toxicants (metals) have an identical mechanism of action. Notably, our previous analysis, the present and other recent studies indicated that Pb and Cd have different respective mechanisms and/or sites of action, which undermined our ability to predict how these metals interacted. Thus, we recommend that future studies examining metal mixture toxicity should also consider and/or determine the mechanism(s) of action for each constituent metal if the goal is to develop reliable predictive models of metal mixture toxicity that can be used for ecological risk assessment.

Toxic outcome drastically altered after exposure to a four-way mixture of Cd, Se, SO_4, and NO_3 in species-specific water (PL)

Sarah Bogart[1], Cindy Meays[2] and Greg Pyle[1]
[1]University of Lethbridge, [2]British Columbia Ministry of Environment

Aquatic organisms are increasingly exposed to multiple contaminants, yet we have little understanding of whether single-contaminant-based water quality guidelines (WQG) can protect aquatic resources when applied to complex mixtures. We assessed the toxicity of Cd, Se, SO_4, and NO_3 and their four-way mixture to *Daphnia magna* and *Hyalella azteca* in 50 (SOFT) and 250 (HARD) mg·L^{-1} as $CaCO_3$ hardness waters. All exposures were at WQG concentrations and species-specific waters were used. Chronic exposures of *D. magna* and *H. azteca* to single contaminants and their four-way mixture in HARD and SOFT water did not elicit mortality greater than acceptable limits for the control (i.e., 10% and 20% for species used, respectively). However, results of acute exposure scenarios were variable and unpredictable. In *Hyalella* tests, the four-way mixture was always toxic and the response was additive, even though mortality in nearly all single-contaminant exposures was below the acceptable control limit (10%). In *Daphnia* tests, the four-way mixture was not toxic in SOFT or HARD water despite Cd being ~95% lethal in HARD water in the single-contaminant exposure. In binary mixture tests, SO_4 was found to ameliorate Cd toxicity in the four-way mixture exposure to *D. magna*. We hypothesize that the observed, acute, mixture toxicity (*Hyalella*) vs. amelioration (*Daphnia*) in HARD water is explained by differences in alkalinity in the species-specific test waters used. Test waters had an alkalinity of 175 and 40 $mg \cdot L^{-1}$ as $CaCO_3$, for *D. magna* and *H. azteca* respectively. Thus, it could be that SO_4-based amelioration of Cd is only possible, at the tested concentrations, in waters of higher alkalinity. Our results show that contaminant mixtures can produce unexpected results due to interactions with major ions in water (e.g., SO_4, HCO_3) and that single-contaminant based WQG may not offer the desired level of aquatic resource protection when applied to a complex mixture scenario.

Nickel and zinc protect against cadmium-induced olfactory toxicity in rainbow trout (PL)

Greg Pyle[1] and Bill Dew[2]
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Freshwater animals rely on chemosensory signals to convey important information directly relevant to their survival, growth, and reproduction. A considerable amount of research attention has recently been directed towards understanding how individual metals interfere with chemosensory function. However, almost nothing is known about the effects of metal-mixtures on olfactory toxicity. In this study, we exposed rainbow trout (*Oncorhynchus mykiss*) for 24 or 96 hours to cadmium, copper, nickel, and zinc, both singly and combined, at concentrations at or below the current Canadian Council of Ministers of the Environment Water Quality Guidelines for the Protection of Aquatic Life. Cadmium exposure induced olfactory toxicity, as measured by electro-olfactography, whereas the other three metals did not. However, cadmium toxicity was completely ameliorated in the quaternary metal mixture. Binary mixtures with major cations (sodium, magnesium, calcium) protected against cadmium toxicity, as well as binary mixtures with nickel and zinc, but not copper. Metallothionein induction could not account for the pattern of metal-induced olfactory toxicity observed in this study. We conclude that metal mixtures lead to competitive interactions at the olfactory epithelium that can ameliorate the toxic effects of a single metal.

Evaluating the interactive effects of waterborne metals (Cd, Cu, Ni and Zn), singly and in binary combination, on fathead minnow (*Pimephales promelas*) reproductive performance (PL)

Melissa Driessnack[1], Ankur Jamwal[1] and Som Niyogi[1]
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The present study examined the interactive effects of metals (Cd, Cu, Ni and Zn) on fish reproduction during chronic waterborne exposures. We used a 21-day fathead minnow (*Pimephales promelas*) reproductive bioassay, where fish were exposed to isotoxic concentrations (15-20% of 96-hour LC_{50}) of each metal, both singly and in binary combinations (Cd and Cu, Cd and Zn, and Cu and Ni). The main objectives were: (a) to determine whether binary metal combinations elicit antagonistic or additive interactions on fish reproductive performance, and (b) to examine whether the responses of key female reproductive and physiological biomarkers (mRNA expression of estrogen receptors (ER-α and ER-β), vitellogenin (Vtg) and metallothionein (Mt), and plasma estradiol) are indicative of metal(s)-induced alterations in reproductive output. Cumulative egg production was found to be the most sensitive reproductive endpoint in our study, which was significantly decreased in all of the single metal exposures except Cd. The binary mixtures of both Cu and Cd, and Cd and Zn produced more than additive effect on cumulative egg production in fish. Interestingly, however, no interactive effect of Cu and Ni was recorded on fish reproductive output, as the cumulative egg production in Cu- or Ni-only exposure was similar relative to that in their mixture treatment. The hepatic mRNA expression of ER-α and ER-β was markedly influenced only by the binary mixture of Cu and Cd, which also corresponded with significantly reduced plasma estradiol level in this treatment relative to that in the respective single metal and control treatments. In contrast, a consistently significant down-regulation of Vtg mRNA expression and up-regulation of Mt expression in fish liver was recorded across all the metal mixture treatments. In general, hepatic Vtg and Mt expression appeared to be better indicators of reproductive effects of binary metal-mixtures in fish. The present study provides new mechanistic insights into the reproductive toxicity of metals, both individually and in binary mixtures.

Industrial Development: Implications for Aboriginal Communities

The importance of community-based environmental monitoring programs in regions containing industrial developments (PL)

Ryan Froess[1], Cassandra Rees[1], Kelly Wells[1], Kevin McCullum[2] and James Irvine[3]
[1]Canada North Environmental Services, [2]Saskatchewan Ministry of Environment, [3]Population Health Unit

The Athabasca region of northern Saskatchewan is home to several Aboriginal communities and also contains five active uranium mining and milling operations. During the past 20 years, there has been extensive community involvement in environmental sampling programs in the region. A key aspect of any successful community monitoring program is that the sampling locations and media are selected based on their importance to the communities and the sampling is completed by, or with, local residents. This helps to build trust between the residents of communities and industrial operators in the region. Traditional Ecological Knowledge (TEK) is also an essential part of these programs. There are currently two such programs being conducted in northern Saskatchewan: the Athabasca Working Group (AWG) Environmental Monitoring Program (EMP) and the Eastern Athabasca Regional Monitoring Program (EARMP). In 1999, the AWG was created under the Impact Management Agreement between Athabasca region communities and uranium companies operating in the area. The AWG is responsible for administration of the EMP, which has been running for 16 consecutive years. The EMP involves testing air and water quality, sediment, fish, plants, and wildlife near each community for contaminants potentially originating from active uranium mining and milling operations. Importantly, the study design and field data collections involve the participation of local residents from each of the communities. In 2011, the EARMP was formed and it is managed under the Province of Saskatchewan's Boreal Watershed Initiative. Two sub-programs, a community program and a technical program, comprise the monitoring framework of the EARMP. The community program, which is funded solely by the province, tests the safety of traditionally harvested foods by collecting and testing water, fish, berry, and mammal chemistry from the seven communities located in the Athabasca region. It also relies heavily on participation of community members for determining sampling locations and collecting the samples. Although the study design for the EARMP community program tries to remain consistent over time (in order to collect a consistent long-term data set) the program is also adaptive and may be refined in response to new information or changes associated with the development in the region, community concerns, or community results.

Investigating concerns raised by Lac La Ronge Indian Band members about the safety of consuming traditionally harvested foods (PL)

Kelly Wells[1] and Ryan Froess[1]
[1]Canada North Environmental Services

The Lac La Ronge Indian Band (LLRIB) is the largest First Nation in Saskatchewan and one of the largest in Canada, with over 10,000 members in six communities and 19 reserves. In recent years, increasing demand on the natural resources sector in northern Saskatchewan has lead members of the LLRIB to question the impacts of mining exploration, mining and milling operations, and abandoned mine sites on the health of their people and environment. Specific concerns were raised about potential health risks associated with eating traditional local foods ("wild foods") that are hunted and gathered in the region. To address these concerns, the LLRIB secured funding in 2013 through the First Nations Environmental Contaminants Program to conduct a one-year community-based research program. The wild foods study was facilitated by Canada North Environmental Services, an environmental consulting firm entirely owned by the LLRIB. A site-specific food frequency questionnaire was developed and band members were hired and trained to conduct interviews with community residents. The results of the survey are based on responses from a total of 104 people from three LLRIB communities. Information on the quantity, type, and location of wild foods consumed by each of three study communities was used to establish a sampling program. Water, sediment, soil, fish, blueberry, duck, grouse, and moose samples were collected from locations near the study communities. In addition, the abandoned Anglo Rouyn copper mine site, located approximately 15 km east of one of the study communities, was identified as a potential concern as access is not limited and evidence of utilization of the site was observed. Water, sediment, soil, and fish samples were collected and tested from the Anglo Rouyn site. Results of the study found that near the communities metal levels did not pose human health risks, with the exception of high lead levels in grouse and moose that were likely due to the use of lead shot. At the Anglo Rouyn site, some metal concentrations were high in water, sediment, and soil samples and it was recommended that access be restricted until the site is determined to be a low risk to human and ecological health by the Saskatchewan Ministry of Environment. Results of the study were communicated to LLRIB members through a report, meetings, public events, and brochures.

Lower Churchill hydroelectric generation project: Dietary surveys and human biomonitoring to support the baseline human health risk assessment program (PL)

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Following its release from the environmental assessment (EA) process in 2012, the Nalcor Energy Lower Churchill Hydroelectric Generation Project (LCHGP) is currently in its construction phase with hydroelectric power generation expected to commence in 2017. As part of fulfilling commitments made during the EA process and meeting various regulatory conditions and requirements (during the EA process and post-EA) that relate to methyl mercury formation, bioaccumulation and methyl mercury exposures and risks, dietary surveys and a human biomonitoring program (hair sampling) were conducted in five Labrador communities in the fall of 2014 and winter of 2015. The data from the diet surveys and biomonitoring program will be used to support a baseline human health risk assessment (HHRA) study. The HHRA will focus on methyl mercury exposures and risks that may result from the consumption of local fish and other country foods of aquatic origin. Diet survey and biomonitoring program results will also provide information on the potential future need for fish or other country food item consumption advisories. This presentation describes the design, implementation and results of the diet surveys and human biomonitoring program.

A community-based cumulative effects monitoring program for the Slave River, Northwest Territories, Canada (PL)

Paul Jones[1], Tim Jardine[1], Lalita Bharadwaj[1], Karl Lindenschmidt[1], Lorne Doig[1], Erin Kelly[2] and Sarah Rosolen[3]

[1]University of Saskatchewan, [2]Government of the Northwest Territories, [3]Aurora College

The Slave River Delta is one of Canada's largest freshwater delta systems. The Slave River is fed by the Athabasca and Peace rivers which flow through Northern Alberta. Increased industrial activity in the Peace/Athabasca river basin has been a source of ongoing concern to First Nation and Métis communities as the Slave River and Delta are part of their traditional way of life. Concerns about water quality and its potential human and wildlife health effects have been frequently expressed by these communities and have been instrumental in the development of the Slave River and Delta Partnership (SRDP), an ongoing partnership of communities, First Nations and Métis leadership, territorial and federal agencies, NGOs and "western scientists". In addition to industrial activities on water quality and the hydrodynamics of the river/delta, there are concerns about the cumulative and interactive impacts of these and other stressors, particularly global climate change. To address the concerns of the communities, we have developed, in partnership with the SRDP, a community-based

monitoring program in partnership with communities, local territorial and federal agencies, NGOs and researchers. The Slave Watershed Environmental Effects Program (SWEEP) is developing a series of indicators that will integrate local and traditional knowledge and western science measures. The findings will be integrated using a Bayesian Belief Network (BNN) and will be freely available to communities and regulators with an emphasis being put on timeliness of delivery and on presenting the results in an easily understood manner. Indicators are being developed to address water quality, hydrodynamics and ice dynamics, wildlife impacts, fish health and quality and human dimensions of the changes being observed. It is hoped that the types of bioindicators being used and the means of integration in the BNN for community presentation will provide an example for the development of other community-based monitoring programs.

Slave River Environmental Effects Monitoring Program (SWEEP) and benthic macroinvertebrates: A community-based approach to monitoring the long-term health of a large northern river (PL)

Lorne Doig[1], Jan Ciborowski[2], Michelle Dobrin[2], Edward Moes[3], Victor Mandeville[4], Frasier McTurk[3], Sarah Rosolen[5] and Slave River and Delta Partnership[6]

[1]University of Saskatchewan, [2]University of Windsor, [3]Deninu School, [4]Deninu Kue First Nation, [5]Aurora College, [6]Various

Residents in the Slave River and Delta area, NWT, Canada, are concerned about the cumulative environmental impacts of various stressors on the health of the Slave River and Delta ecosystem. One approach is to monitor the benthic invertebrate community, the composition of which changes markedly in response to pollution or other stressors. The goal of this project component was to develop a community-based process to monitor the benthic invertebrate communities of the Slave River and Delta. Hester-Dendy samplers were deployed for 2-8 weeks in the Slave River near Fort Smith and in the Jean River and Steamboat channel (part of the Delta) to determine optimum deployment time. The samplers were colonized by a diversity of animals including those considered sensitive to pollution (e.g., mayflies, stoneflies, and caddisflies). With basic training and guidance, students of Deninu School (grades 10 to 11), Fort Resolution, were able to identify and count the animals in the 6-week interval samples. With assistance from someone familiar with benthic invertebrate taxonomy, the students correctly identified 95.2% ± 3.8% of the invertebrates (n=7) relative to expert identification. Animal abundance (animals/m^2) and taxa diversity equilibrated after 4 weeks, and was highest in the Delta (ranging from 609 (Fort Smith) to 3740 (Steamboat channel) animals/m^2). Taxonomic experts discerned more taxa than the students, mainly due to the experts' familiarity with more genera of diverse families. Overall, Hester-Dendy sampling was an effective means for the community to assess differences in the community composition and abundance of benthic invertebrates of the Slave River and Delta.

Predicting mercury distribution in the Slave River and Delta using geospatial modeling (PL)

Lorne Doig[1], Anna Meissner[1], Tim Jardine[1], Paul Jones[1], Lalita Bharadwaj[1] and Karl-Erich Lindenschmidt[1]
[1]University of Saskatchewan

Intensive industrial activities upstream of the Slave River and Delta, NWT, have raised concerns regarding regional contamination. Environmental monitoring can help to assess the status and trends of contaminants; however, prior to field operations, geospatial modeling has the potential to identify worst-case areas of contamination. Fluvial systems contain many different habitats due to a wide range of flow, topography, and geology. Based on the number and length of bends, the speed of flow, water depth, and other features, satellite imagery can be used to group reaches of the river into different geomorphological response units (GRUs). Geomorphology affects the river's flow, which influences grain-size distribution and the resulting spatial distribution of associated metals. Using satellite imagery of the Slave River and Delta, this study assessed the association of mercury (Hg) with pre-defined GRUs and the effectiveness of this approach for understanding potential hot-spots of Hg deposition and availability. Surface sediment samples were collected from six pre-defined GRUs and analyzed for particle size distribution, Hg and organic carbon content. Concentrations of Hg at all sites were well below the Interim Sediment Quality Guideline of the Canadian Council of Ministers of the Environment (i.e., less than 170 $ng{\cdot}g^{-1}$), and were strongly correlated with percent clay content ($R=0.918$, $p<0.001$, $n=60$) and total organic carbon ($R=0.943$, $p<0.001$, $n=59$), both of which co-varied ($R=0.858$, $p<0.001$, $n=59$). GRUs that were less sinuous, wider, and deeper in the main channel contained the highest sand content (i.e., lowest clay content) and therefore had the lowest concentrations of Hg. Grain size distributions (and hence Hg concentrations) were variable within the smaller distributary channel GRUs due to the presence of consolidated clay beds at some stations. Although the constituent materials at these sites were fine-grained and easily erodible, consolidation decreased the susceptibility of the sediment bed to erosion, allowing fine materials to occur in faster flowing waters alongside heavier but non-cohesive materials such as sand. Overall, geomorphological modeling successfully identified best-case sites (i.e., those sites largely devoid of clay). However, the subtle distinctions used to define the distributary channel GRUs do not appear to predict clay distribution and associated Hg.

Author Index

A

Adams, W. 8
Akhter, E. 30
Akhter, F. 6
Alderman, S. 82
Alessi, D. 88, 90
Alharbi, H. 92
Alisauskas, R. 122
Alliot, F. 13
Alves Beese, L. 15
An, S.-J. 49, 50
Anaquod, P. 112
Anas, M. 86
Anderson, J. 25
Angelow, R. 49, 50
Ankley, G. 133, 136
Antunes, P. 95
Arciszewski, T. 111
Arlos, M. 74
Arnold, M. 125
Audet, C. 89
Awuah, K. 97

B

Backus, S. 10
Bagatim, T. 69, 71
Bahamonde, P. 72
Bailey, H. 115
Baird, J. 5
Baker, J. 105, 109, 115
Balakrishnan, V. 102
Baldwin, N. 93
Balicki, B. 107
Barrett, T. 7, 111
Bartlett, A. 80, 102, 140
Basu, N. 3, 67, 123
Baulch, H. 29, 96
Baxter, L. 138
Beitel, S. 22, 27, 28, 42, 43
Beking, M. 55
Benson, S. 114
Bernhardt, E. 38
Berninger, J. 133
Best, C. 59
Bettles, C. 5
Bharadwaj, L. 151, 153
Bianchini, K. 125
Bilodeau, J. 93
Birceanu, O. 65
Blackwell, B. 136
Blais, J. 93
Bogart, S. 83, 146
Bourges, C. 13
Boyle, D. 32
Brack, W. 27, 51, 52
Bradley, D. 76
Bragg, L. 69, 72, 73, 74
Brain, R. 138
Brandenburg, J. 67
Brandt, J. 38
Bresee, K. 89
Bright, D. 98
Brix, K. 144
Brousseau, P. 74
Brown, G. 58
Brown, L. 80, 140
Brunswick, P. 80
Bugden, M. 116
Burnett-Seidel, C. 115
Burton, A. 76

C

Cabana, G. 74
Calfee, R. 62
Carriere, G. 8
Cavallaro, M. 141
Cave, M. 100
Cessna, A. 11
Challis, J. 77
Chang, M. V. 64
Chapman, P. 7
Chen, Y. 31
Chevreuil, M. 13

Chow, S. 60
Ciborowski, J. 87, 131, 152
Clark, R. 6
Clayden, M. 9
Clemow, Y. 145
Codling, G. 84
Coffin, M. 13
Colette, T. 133
Connors, K. 116
Costa, E.-J. 35
Cottin, G. 134
Courtenay, S. 13
Cousins, M. 97
Couture, P. 74
Crawford, S. 118
Crémazy, A. 144

D

D'Silva, L. 29, 96
Dagher, E. 113
Daley, J. 76
Davies, M. 85, 105
Davis, J. 133
de Boer, D. 85
de Solla, S. 10, 66, 122, 127, 140
deBruyn, A. 35, 103
Denslow, N. 133
DesJardins, M. 105
Desportes, A. 13
Desrosiers, M. 74
Deutschmann, B. 27
Dew, B. 147
Dhiyebi, H. 69, 74
Di Giulio, R. 38
Di Paolo, C. 28
Dietz, R. 123
Dillman, B. 57
Dindia, L. 65, 82
Dings-Avery, C. 87
Dixon, D.G. 15, 16, 50
Dobrin, M. 152
Doering, J. 42, 43, 135
Doig, L. 19, 29, 37, 81, 96, 151, 152, 153
Dorais, V. 55
Doucette, J. 2
Douville, M. 134
Dowsley, B. 113, 115
Driessnack, M. 148
Dubetz, C. 12
Dudley, L. 114
Duffy, M. 8
Dumanski, M. 46
Dupuis, A. 12
Durante, J. 25
Durhan, E. 133
Dwyer, J. 103

E

Eadsforth, C. 43
Eid, E. 133
Eisner, B. 27, 28, 42, 43, 135
Ekman, D. 133
Elliott, B. 55
Elphick, J. 105, 115
Elvidge, C. 58
Engelking, S. 142
Evans, M. 85, 86

F

Farrell, A. 82
Felix, L. 19
Fenton, H. 49, 50
Fernandes, S. 114, 118
Fernet, J. 107
Ferone, J. 55
Festarini, A. 116
Finley, M. 9
Flahr, L. 138
Flynn, S. 88, 90
Fobil, J. 3
Folkert, E. 88, 90
Fontanay, S. 26
Foote, A. L. 131
Fournier, M. 24, 74
Frank, R. 80
Fraser, B. 8
Fraser, C. 34
Friesen, M. 139
Froess, R. 117, 149, 150
Fudge, L. 151
Fuzzen, M. 72, 73, 74

G

Gagné, F. ... 69, 74, 75
Gagné, J.-P. ... 89
Gagnon, D. ... 35
Gainer, A. ... 130
Gallant, M. ... 20, 93
Gamberg, M. ... 10
Gauthier, C. ... 69, 74
Gauthier, I. ... 120
Giesy, J. . 4, 19, 22, 23, 42, 65, 69, 71, 79, 84, 91, 135, 137
Gillespie, K. ... 34
Gillio Meina, E. ... 82
Gillis, P. ... 10, 80, 102, 140
Gillis, T. ... 82
Gilroy, È. ... 10
Giraudo, M. ... 134
Gobas, F. ... 26
Goertzen, M. ... 47
Goldblatt, R. ... 47
Goss, G. ... 19, 32, 88, 90
Graves, S. ... 16
Green, D. ... 8
Grote, M. ... 51
Guan, M. ... 91
Guchardi, J. ... 25
Gurney, K. ... 122

H

Haakensen, M. ... 37, 131, 132
Hala, D. ... 134
Hale, B. ... 97
Halyk, L. ... 89
Hanson, M. ... 45, 53, 54, 77, 138
Hanson, S. ... 22, 69, 71
Harbi, H. ... 79
Harrington, J. ... 129
Hart, D. ... 46
Hazell, M. ... 131
He, Y. ... 88, 90
Head, J. ... 67
Headley, J. ... 11, 141
Hecker, M. ... 19, 21, 22, 27, 28, 35, 39, 42, 43, 69, 71, 75, 135
Hedges, A. ... 140
Heerema, J. ... 70
Heide, T. ... 42
Helbing, C. ... 70
Hennessy, R. ... 100
Hersikorn, B. ... 31
Hewitt, M. ... 80
Hicks, K. ... 73, 74
Hill, A. ... 4, 23
Hill, R. ... 105
Hobson, K. ... 6
Hogan, N. ... 1, 17, 20, 69, 71, 89, 93
Holdway, D. ... 25
Hollert, H. ... 27, 28, 42, 52
Hontela, A. ... 69, 71
Hood, A. ... 7
Hosmer, A. ... 138
Houck, K. ... 136
Houde, M. ... 134
Huber, G. ... 101
Huebert, D. ... 109, 110
Huffman, D. ... 114
Hughes, K. ... 122, 127
Hughes, S. ... 43
Huntsman-Mapila, P. ... 95

I

Inglis, C. ... 55
Irvine, J. ... 149

J

Jagla, M. ... 55
James, K. ... 100
Jamwal, A. ... 41, 148
Janz, D. ... 8, 22, 36, 39
Jardine, T. ... 8, 21, 151, 153
Jatar, M. ... 100
Jeffries, D. ... 86
Jegede, K. ... 97
Jensen, K. ... 133
Jimmo, A. ... 143
Johnson, C. ... 40
Jones, P. ... 4, 19, 22, 23, 69, 71, 84, 151, 153

K

Kahl, M. ... 133
Kansas, J. ... 107
Keating, J. ... 86

Keatley, B. 12
Keene, J. 109
Kelly, E. 4, 84, 151
Kennedy, C. 82, 142
Kidd, K. 9, 16, 41, 75
Kilgour, B. 85, 111, 115
King, L. 10
King, M. 40
Kinniburgh, D. 124
Klemish, J. 83
Klinck, J. 10
Knapp, C. 77
Knysh, K. 13
Köllner, B. 1
Krohn, R. 30
Kuo, J. 55
Kuzma, J. 65
Kwamena, N.-O. 113

L

Labadie, P. 13
Lacaze, É. 74
Laird, A. 97
Lajeunesse, A. 74
Lam, Y. 105
Lamb, E. 100
Langlois, V. 66
Lannoo, M. 83
Lari, E. 61
Leal, F. 18
Leblanc, J. 12
Lee, K. 105
Lee-Jenkins, S. 121
Lemieux, H. 100
Leon, L. 118
Lescord, G. 9
Leslie, J. 45
Lewis, C. 61
Liber, K. 29, 37, 81, 82, 92, 96, 118, 141
Lin, F. 82
Lindenschmidt, K. 151, 153
Lintott, D. 101
Little, E. 62
Lobson, C. 54, 77
Lucas, B. 105
Luong, K. 77

M

MacDonald, N. 49, 50
Machtans, H. 7
MacLatchy, D. 74, 77
Madden, P. 151
Main, A. 11
Malaj, E. 51
Maloney, E. 141
Mandeville, V. 152
Manek, A. 57, 70
Mankidy, R. 91
Marcus, E. 105
Marjan, P. 73, 74, 77
Markert, N. 27
Markwart, B. 37
Marlatt, V. 142
Martin, J. 88, 90, 91
Martin, P. 127
Martinovic-Weigelt, D. 133
Martyniuk, C. 74, 77
Massé, A. 36
Matthews, A. 31
McAvoy, D. 76
McCallum, E. 63
McCann, E.K. 73, 74
McConkey, B. 65
McCullum, K. 8, 107, 149
McDougall, M. 45
McInnis, R. 10, 140
McKee, M. 113, 115
McKernan, M. 110
McLoughlin, P. 126
McMaster, M. 72, 73, 74, 80
McPhee, L. 89
McTurk, F. 152
Meays, C. 57, 146
Mebane, C. 8
Meegahage, B. 86
Mehinto, A. 133
Meissner, A. 153
Melnyk-Lamont, N. 59
Menard, K. 131
Michel, N. 6, 11
Mihok, S. 115
Milani, D. 102

Milne Wilkinson, L. 50
Moate, A. 21
Modares, S. 142
Moes, E. 152
Mohaddes, E. 61
Monabbati, M. 114
Moody, M. 98, 99
Moon, T. 64
Moore, D. 48, 53, 122
Morandi, G. 91
Morgan-Gray, T. 50
Morrissey, C. 6, 11, 125, 138, 141
Mothersill, C. 116
Muldoon, B. 17
Mundy, L. 6
Munkittrick, K. 16, 41, 111
Munro, K. 104
Murphy, B. 112
Muscatello, J. 36, 125

N

Nadella, S. 145
Naile, J. 43
Nelson, J. 98
Newstead, D. 125
Nicholson, R. 46
Nilsen, J. 109
Niyogi, S. 41, 144, 145, 148
North, M. 124
Norwood, W. 16, 50
Novak, L. 95

O

O’Driscoll, N. 9
Ohiozebau, E. 4, 23, 84
Orr, P. 34, 116
Ortega, V. 32
Ouellet, M. 120

P

Pagano, N. 28
Palace, V. 25, 45
Park, B. 12, 25
Parrott, J. 80
Pasternak, J. 55
Pathammavong, P. 16
Patterson, S. 35, 39
Pauli, B. 6, 93, 120, 121
Peery, H. 116
Pelletier, E. 89
Peng, H. 79, 135
Pereira, A. 91
Peru, K. 11, 141
Peters, R. 53, 100
Petrie, S. 127
Pettem, C. 39
Phalen, L. 1
Pham, S. 117
Philibert, C. 61
Philibert, D. 61
Phillips, H. 118
Pippy, B. 41
Pittet, V. 37, 131, 132
Poirier, D. 48
Princz, J. 100
Prosser, R. 102
Puglis, H. 62
Pyle, G. 57, 60, 61, 70, 83, 146, 147

R

Raine, J. 31, 43
Raqib, R. 30
Rees, C. 2, 149
Rendas, M. 95
Rentz, J. 40
Repaso-Subang, T. 151
Revel, M. 24
Richardson, S. 121
Rickwood, C. 40, 95
Robidoux, P. 24
Robins, T. 3
Robinson, C. 5
Robinson, S. 121
Rodrigue, J. 120
Rosolen, S. 151, 152
Ross, B. 12
Rozon-Ramilo, L. 109
Russel, C. 116

S

Saint-Louis, R. 89
Sarauer, B. 22

Satkunam, M. 116
Saunders, D. 65, 137
Saunders, L. 26
Sauvé, S. 74
Schäfer, R. 51
Schiffer, S. 81
Schmidt, B. 28
Schneider, U. 103
Schock, D. 6
Schroeder, A. 136
Schultz, D. 22
Schummer, M. 127
Scroggins, R. 44, 95, 98, 99, 100
Seiler, T.-B. 28
Semper, C. 89
Senn, L. 28
Serben, K. 49, 103
Servos, M. 69, 72, 73, 74, 75, 77
Shang, D. 80
Sharpe, R. 7
Shephard, B. 57
Shpyth, A. 46, 114
Sibbald, C. 109, 110
Sibley, P. 48
Siciliano, S. 53, 89, 97, 100, 130, 143
Simko, E. 139
Skatter, H. 107
Slattery, S. 122
Slezak, B. 49, 50
Smits, J. 3, 30, 124
Smulders, C. 43
Solomon, K. 48, 138
Somers, K. 111
Sonne, C. 123
Sonnenberg, H. 104, 109
Soos, C. 6
Soron, M. 31
Stafford, J. 32
Steeves, K. 69, 71
Steinkey, D. 57
Stetefeld, J. 45
Stewart, K. 143
St-Martin, G. 142
Struger, J. 10, 140
Stuart, M. 116
Stutchbury, B. 138
Su, B. 116
Sun, J. 65, 79, 135

T

Tang, J. 114
Tang, S. 22, 39, 135
Tanna, R. 72
Taylor, L. 44, 95, 100
Tendler, B. 4, 23, 84
Tétreault, G. 72, 73, 74
Thalmann, B. 27
Thompson, A. 59
Tierney, K. 61
Tlili, K. 13
Tremblay, R. 89
Trudeau, V. 64, 121
Tse, T. 19
Turner, C. 76

V

van den Heuvel, M. 1, 13
Van Der Kraak, G. 74
Van der Vliet, L. 100
Van Kessel, A. 21
Vandenberg, A. 30
Vargas, J. 107
Vengosh, A. 38
Vignet, C. 80
Vijayan, M. 59, 65
Villeneuve, D. 133, 136
Virgl, J. 108
Von der Ohe, P. 51

W

Wallace, S. 66
Walsh, S. 116
Wang, Z. 80
Warren, L. 128
Weber, L. 39, 57
Webster, M. 118
Weech, S. 34
Weisener, C. 87, 129
Wells, J. 151
Wells, K. 2, 149, 150
Weseloh, C. 122
Whale, G. 43

Wheater, H. 19
White, K. 92
Wickstrom, M. 53, 100
Wijaya, J. 105
Wilcockson, J. 105
Wilkie, M. 145
Williams, R. 151
Willis, R. 151
Wiramanaden, C. 116
Wiseman, S. 21, 22, 39, 42, 43, 65, 69, 71, 79, 91, 135, 137
Wissel, B. 86
Witt, J. 16
Wobeser, G. 139
Wolfe, J. 76
Wong, C. 53, 77
Wood, C. 122, 144, 145
Wood, S. 139

Y

Yang, C. 80
Yao, K. 55
Yuan, H. 69

Z

Z'Graggen, M. 49, 50
Zdunich, K. 112
Zee, J. 35, 39
Zhang, X. 91